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BLASTOPORE AND SPINA BIFIDA
A COMPARATIVE MORPHOLOGICAL, TERATOLOGICAL
STUDY ON MALFORMED FROG'S EGGS

Oscar Hertwig

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BLASTOPORE AND SPINA BIFIDA

A Comparative Morphological, Teratological
Study on Malformed Frog's Eggs

O. Hertwig

ABS

ABSTRACT. This work is a comparative morphological, teratological study on malformed frog's eggs. The author studies disorders of the fertilization process; disorders in the gastrulation process and the malformations resulting from these disorders. A critical examination and evaluation of the findings of the author's studies are given; he also discusses similar malformations in other classes of vertebrates. Malformations in the three higher vertebrate classes are studied and the findings are applied to basic questions of vertebrate animal morphology. The coelmic theory and relation of the blastopore to different forms of malformations are discussed. The author also gives conditions under which multiple rudiments can arise from a single egg cell. Figures are presented and discussed.

In order to go more deeply into the question, which is still wrapped in darkness, of whether malformations can be produced through polyspermy, this Spring I again took up experiments on eggs of *Rana Temporaria*, experiments which I had begun years ago but discontinued. I produced polyspermy in frog's eggs in two ways, after chemical methods failed to yield the desired result. First, I removed the uterus filled with eggs from the abdominal cavity of the female and placed it for 2-4 days in a moist chamber in order to cause damage by this treatment and to obtain results similar to those one obtained with echinoderm eggs which have been allowed to lie in sea water for 1-2 days after the emptying of the ovary, before the semen is added. It is surprising how little the frog's eggs suffer in general from this treatment. For even on the third and fourth days a large portion of them develops in a normal manner; another portion shows irregularities in the process of cleavage from which polyspermy might be inferred; finally, a third portion does not develop, even if spermatozoa have perhaps penetrated the yolk.

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* Numbers in the margin indicate pagination in the foreign text.

A second procedure for obtaining polyspermy consisted of separating the frog pairs from one another and isolating the males from the females for 4-6 weeks. When the eggs which have entered the uterus are not deposited for so long past the normal time, they are likewise damaged, as has also been observed in echinoderms and fishes (trout); they come into a condition which I have labeled as overripeness. Here too, more or less frequent derangements in the process of cleavage appear beside eggs which are dividing normally. /354

In different cultures obtained in this way I did not chance upon a single multiple formation, although I had directed my special attention to this. In this respect, my experiments had remained without the desired result. On the other hand, however, in the culture glasses, many eggs attracted my attention, which had developed pathologically in a peculiar manner and were therefore examined in detail to determine whether they had any connection with the question which was interesting me. They were distinguished by a now more, now less wide potency of the blastopore up to late stages of development.

To date, there has been only one more detailed note in the literature concerning such malformations of the frog's eggs by Roux in his contributions to the mechanism of development of the embryo. Roux [61] describes the malformation under the title of asyntaxia or diastasis medullaris.

"Both medullary folds," he remarks, "are situated far apart, while they occupy the lateral margins of a longitudinal, almost level plate representing the embryo; under each medullary fold there is present through the assembly of a mere 3 to 4 cells, a fine, but likewise round semichorda lateralis, which is well characterized in cross-section. Similar but smaller divergence of the medullary folds was also found to occur repeatedly only partially, especially in the areas of the posterior half of the spinal cord. In this case the presence of the endoderm was detectable in sections; on the other hand, however, it could easily be established by repeated observation on the living egg that the large cleft between both medullary folds represents the blastopore or the remainder of it". "In cases of asyntaxia medullaris, in the middle and caudal portion of the embryo only, I subsequently saw more frequently a further approximation of the medullary folds take place, actually more on the caudal side, so that finally only a hole still remained in the middle of the length of the embryonic medullary tube which, however, later on became closed as well. Therefore, this was only a delay in the downgrowth of the half dorsal plate on either side from the equator of the egg, while the qualitative differentiation produced the medullary folds before fusion of the halves of the dorsal plate without being retarded by the former. This form of diastasis medullaris and the derivation of the cleavage space from the blastopore illustrates most clearly an analogy of the formation of the embryonic formation in amphibians with that in the fishes and thus connects also the asyntaxia medullaris with the "delay in joining of the halves of the germinal ring for the formation of the central and posterior embryonic anlage" as described by Rauber for the bone fishes." /355

Since my cultures furnished a very plentiful supply of malformations, so that I was able to isolate about 100 of these in a short time, I preserved them at different intervals after fertilization in 1% chromic acid with addition of 0.2% acetic acid. After sufficient hardening the gelatinous envelopes were removed by careful shaking in Javelle solution as recommended by Blochmann [2b] and the embryos thus exposed were preserved in 85% alcohol. In this manner I obtained a very complete series of malformed frog's eggs in different stages of development which, in spite of diverse variations in the degree of malformation, still presented a quite typical appearance. In this I found encouragement for further investigation. With little magnification, the individual eggs were closely examined from different sides and sketched with the *camera lucida*. Thereupon they were dissected into series of cross-wise, sagittal, or frontal sections as appeared desirable in the particular case. In preparing the sagittal sections only one half was used in most cases; the other half could then still serve for a series of cross-sections. Embedding in paraffin was done according to the procedure suggested by Oscar Schultze [62] which I also found to be suitable.

The results of the rather extensive investigation are reported in the following three chapters. Of these the first Chapter deals with disorders of the process of fertilization; the second deals with monstrous development of eggs where larger or lesser portions of the yolk remained undivided; the third deals with disorders of the process of gastrulation which lead to defective closing of the blastopore and as a consequence thereof to a series of malformations.

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1. Disorders of the Process of Fertilization

The egg material taken from a female frog which had been isolated for several weeks and killed on March 14 was examined for irregularities in the process of fertilization. The eggs remained two more days in the uterus and were fertilized on March 16. Four hours after fertilization the majority began to cleave in a completely regular manner. However, a smaller portion showed an indication of disorders of the normal process upon magnification with a magnifying glass. On the black pigmented half of the egg there appeared unusual, irregular grooves which led not to a symmetrical dissection of the yolk but only to a demarcation of smaller and larger fields (Plate 16, Fig. 32-35). In a word, the pictures developed which have already been more precisely described by Born [4] and for which he introduced the name baroque cleavage. Twenty-five eggs which showed deviations from the normal fertilization pattern, were immediately isolated in numbered watch glasses and the condition of each was quickly recorded.

Even with this small stock it could be established that some characteristic states recurred more frequently. Accordingly, at least two groups of abnormally cleaving eggs may be distinguished for the time being.

In one group (Figs. 34 and 35) several main furrows appeared in the black half of the egg, ones which generally met in one place and again formed the starting point for branches going off laterally. Further, the meeting of

several furrows marked off several smaller, irregular fields on the animal polar surface of the egg, from which shorter or longer furrows extended towards the vegetative pole. After some time these usually divided even the light half of the egg into somewhat larger fields.

The second group of eggs (Figs. 32 and 33) is characterized by the appearance of a ring shaped furrow which delineates a sometimes smaller, sometimes larger circular field on the animal pole. Since, soon after its appearance, the furrow probably passes completely through the yolk mass, it cuts off a smaller disc from the egg which is situated on the animal pole and perches on the much larger remaining portion like a calotte. Soon after, radial furrows extend from the circular furrow toward the vegetative pole and dissect the remaining egg mass.

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The eggs which were thus irregularly cleaved in different manner were further cultured in their small containers for several days. After further division into small cells had taken place the larger portion of them stopped in their development and to me appeared to show signs of dying in that a grayish white coating formed on the vegetative pole. The remaining eggs entered the gastrula stage. The medullary folds arose in regular fashion on the fourth day after fertilization. Accordingly, an apparently normal development had still come about in these cases. Almost all of these eggs belonged to the second group. It should be possible to decide by a more extensive investigation directed especially to this problem whether this is definite relationship or only a coincidence. This would not be without interest since the abnormal fertilization process could have been caused only by polyspermy and because the different variations of Baroque cleavage will depend on whether two, three, or more spermatozoa have penetrated into the yolk and in what number they have combined with the egg nucleus or have remained in the yolk isolated by themselves. That such events must take place here, we may conclude first, from the investigations of Born [4] concerning polyspermy and Baroque cleavage in amphibian eggs, and further, especially from the abnormal fertilization and cleavage processes of Echinoderm eggs observed by Fol [17], my brother, and myself [23, 27].

2. Monstrous Development in Eggs in Which More or Less Large Portions of the Yolk Remain Undivided. (Plate 20, Figs. 21-27)

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A larger number of frog eggs were divided into a series of sections which presented rather peculiar and surprising appearance. The main mass of the yolk, or only half or a still smaller portion was not divided into cells, while the remainder consisted of small cells which had arranged themselves sometimes even in the form of germinal layers, etc. In all these cases the yolk remaining uncleaved had also changed its character. It was permeated by numerous smaller and larger vacuoles which were filled with a clear fluid and often lay so close together that they were separated from one another only by thin septa like the bubbles of lather [Figs. 23, 27 va.]. Often even larger and then irregularly contoured vacuoles had been formed by the thin septa breaking down between several vacuoles (Fig. 21 va). Moreover, numerous small bubbles appeared in the altered yolk which were

covered with a dark pigment and at times also showed radiation in their environment (Fig. 23 p). I do not hesitate to consider them as nuclei stemming from numerous spermatozoa which penetrated into the egg or possibly also from division of the sperm nuclei. I was confirmed in this opinion particularly by the observation of individual isolated cells in the yolk which was permeated with vacuoles. However, I found these only in some cases (Fig. 21, 22, 24, 27z'), while they were absent in others. Fig. 25 shows such a cell (z') at somewhat higher magnification. A nucleus situated in a pigmented area is surrounded by a covering of yolk substance which is sharply set off by a smooth contour from the remaining vacuole-containing yolk all around. Sometimes several cells occur together in one group (Fig. 21z').

On the animal surface of this peculiarly altered egg a cleavage process occurred sometimes in lesser, sometimes in greater extent; it had given rise to a layer of small embryonic, partially pigmented cells, which rest on the vacuole-containing yolk in a manner comparable to the germinal disc of a meroblastic egg. I came across a very small degree of cell formation in the case of egg a' (Fig. 23). Here, nearly one-fourth of the surface of the yolk was covered by a simple layer of small cubic cells. On the periphery of the disc the surface of the egg was divided by furrows into small fields which contained nuclei but were still connected to the remaining yolk mass. Also, a small group of cells had formed on this object at some distance from the main disc.

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In two other eggs (Fig. 21 and 22) a disc three to five cells thick had been formed through partial, irregular cleavage. In one case (Fig. 21) a sort of cleavage cavity (fh) lay beneath the disc, a large hollow space about the origin of which I cannot make any definite statement. The possibility that it may be formed by the fusion of many individual vacuoles should be kept in mind. The entire egg may be considered as a blastoderm whose vegetative half consists of undivided yolk mass while the animal half has separated into cells. In Fig. 21, groups of small cells (z') further deserve special attention in addition to completely isolated cells which are found scattered in the vacuole-containing yolk.

If we question the origin of these monstrous forms which protrude so completely from the bounds of the normal process of development of amphibian eggs, then I for one do not doubt for a moment that they have been produced by polyspermy. According to my findings, the entire process may take place in the following manner. The excitability of the protoplasm of the eggs was reduced as a result of overripeness and other injurious influences. The consequence was polyspermy. The sperm nuclei, which penetrated in greater number and of which perhaps one or several have fused with the egg nucleus, produced baroque cleavage at the animal pole of the egg, since greater amounts of protoplasm have accumulated there. Corresponding to the multitude of nuclei, the cortex of the egg divided similarly into several irregular fields as described above. With further increase of the nuclei these fields are then divided into smaller fields which are subsequently cut off from the underlying yolk and transformed into embryonic cells.

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In the vegetative half of the egg there is no decisive cell formation because in this half, because of the protoplasm which is present only in a lesser amount and with the damage to the latter, to some extent the moving forces for the preparation of a process of cleavage are too insignificant. Thus, through pathological conditions a situation is created here similar to that which normally exists in meroblastic eggs where the excessive growth of the yolk mass has separated the egg cell into a divisible portion rich in protoplasm, and a non-participating portion lower in protoplasm. Only as an exception here and there has some yolk mass ever surrounded a sperm nucleus and separated as an independent cell from the remaining mass.

How the vacuoles in the unfertilized portion of the yolk come into being remained unclear to me since I had not specially directed my investigation to this question. Two possibilities should be considered. First, the vacuoles might form by liquefaction of the yolk and might be connected with disintegration of the same. On the other hand, however, they might also have been produced by sperm nuclei which have multiplied by division and subsequently transformed into large bubbles by taking in nuclear sap and in the process have also entered into fusion with one another.

In the case of pathological Echinoderm eggs, I often observed [23] such swelling of small nuclei to large bubbles, and fusion of the same. Whether the first or the second mode of formation occurs should be decided by an investigation directed especially to this question.

Roux [61] has observed vacuoles and nuclei in the non-furrowed yolk of frogs eggs when he destroyed one of the first two blastomeres by piercing it with a heated needle. After some time the vacuoles appear in the yolk of the treated cell and "often lie so thick that in cross-sectional view they are separated from another in places only by a fine protoplasmic thread, and sometimes only vestiges of these structures remain which, considered bodily, represent cleavage membranes so that communication or fusion of the vacuoles becomes readily apparent". Moreover, Roux still found nuclei in the vacuole-containing yolk which he attributed to the cleavage nucleus of the treated blastomere. In this case then, the nucleus would have partially multiplied while the yolk could no longer react because of the damage from piercing.

On this occasion, Roux also mentions results similar to those which I have just reported. "Towards the end of the spawning period in frogs partial development of the egg occurs sometimes without an operation. In several such eggs," he writes, "only three or four cells were present that had the nature of cells in the transition stage from the morula to the blastula state, while the entire remaining egg mass was not organized into cells but rather, was permeated in places with the forms of abnormal nuclei described above and in large sections also with young, normal appearing nuclei in large numbers. Sometimes the egg substance was so heavily vacuolated in the immediate vicinity of the few normal cells that a passage of nuclei therefrom cannot even be considered". Roux thinks that multinucleosis from polyspermy cannot be considered in these cases, even though

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it occasionally occurs toward the end of the spawning period. Rather, he assumes that the cleavage nucleus has formed partly abnormal, and partly apparently normal derivatives. I hold the opposite view and maintain that the peculiar complex of phenomena, including Baroque cleavage, partial cell formation which reminds one of partial cleavage, the occurrence of nuclei and, here and there, of isolated cells in the vacuole-containing yolk, is caused entirely by polyspermy of damaged eggs.

Partially divided eggs are capable of further development especially if only half or only a third of the yolk has remained undivided. Among my stock I found eggs in which the cells had united in a regular fashion to form germ layers, and in which even a kind of gastrulation had taken place; and finally also eggs in which there was formation of the chord and medullary plate (Figs. 24, 26, 27). /362

A pathological gastrula is pictured in Fig. 24. One half of the egg is transformed into a vacuole-containing yolk mass which in one place, where it joins the cellular portion, also encloses nuclei and isolated cells. The other half which is divided into cells is separated by a clear line of demarcation from the portion of the egg which has remained undeveloped. It resembles a small gastrula. On one side of the archenteron lies a conspicuous band of large yolk cells; the opposite wall is thin and is composed of small pigmented cells which are arranged in two layers and pass over each other by turning over at the lip of the blastopore.

Eggs a¹² and a¹¹ provided me with two peculiar malformations in which about half of the substance remained undeveloped (Fig. 26, 27). In both eggs, in the middle between vacuole-containing yolk mass and the cellular half there was a large hollow space (fh) which I take to be the remainder of the cleavage cavity. In Fig. 27, the cellular portion conceals a small invagination cavity, the primitive digestive tract (ud). To the left, a cluster of large yolk cells form its border, to the right and downwards a mass of small pigmented cells separated into several germinal layers. On this mass, a thin deposit of cells is clearly marked off as ectoderm. Where the ectoderm changes into the cell layer lining the primitive digestive tract, it is considerably thickened and transformed into a structure (mp) which is deceptively similar to the cross-section of a medullary plate. Since in the following chapter we will become acquainted with malformed frog's eggs in which the nervous system attaches itself in a corresponding manner to the lip of the blastopore (compare Figs. 8, 9, 15, 16 in Plate 17), I do not hesitate to indicate as medullary plate the portion designated by (mp). To the right, the gastrula is separated partially by a fissure and partially by a line of demarcation from the undivided remainder of the egg; to the left a thin cellular lamella passes over into the vacuole containing yolk, which here also represents only a thin layer and promotes closure of the cleavage cavity (fh) mentioned above. /363

On the side that is turned upward, Fig. 26 shows a conspicuous vacuole-containing yolk mass, below it a cellular embryo divided into two layers. Between the two there is a spacious cleavage cavity (fh) marked off all

around. On the right hand, again in the place where the cellular portion grades into the vacuole-containing portion, a small invagination (ud) has formed. At the lip of the blastopore the outer layer transforms into the inner layer and has thickened again, as in the case previously cited, to form a medullary plate (mp) which here, however, is only slightly curved inward to form a groove. Both to the right and left of the cleavage cavity, the cellular embryo and the undivided remainder of the yolk stand in direct connection with each other.

Similar results were further obtained in a series of sections through several eggs.

Finally, I also came across eggs in which the layout of the chord and primitive segments could be more or less clearly detected alongside an undivided, generally smaller, yolk residue. However, the investigation of all these pathological eggs is attended by difficulties because very little can be seen on the eggs externally, and therefore the direction in which the sections should best be made cannot be determined on embedding in paraffin.

3. Disorders In the Process of Gastrulation Which Lead to Defective Closure of the Blastopore and Consequently to a Series of Malformations

Disorders in the process of gastrulation can become the cause of quite heterogeneous, abnormal conditions. In order to consider these in systematic order it appears practical to divide them into three groups.

In the highest degree of disorder, the blastopore remains open along its entire length at a time when the medullary plate, the chord and several primitive segments have already been formed in the embryo. From this point there exist all conceivable transitions to lesser degrees of disorder. One finds malformed embryos in which only a part of the head or the entire head, or in addition to it a greater or lesser neighboring section of the torso have developed in a normal fashion, but were on the dorsum a corresponding large part of the blastopore in front of the caudal end has remained open. Consequently, a sometimes larger, sometimes smaller portion of the yolk is clearly visible and protrudes to the outside like a kind of yolk plug similar to a Ruskomian anus. /364

Finally, in a third group, I place the least conspicuous disorders in the closure of the blastopore. Here, the entire dorsal region of the embryo is well-developed and separated into nerve tube, chord and primitive segments. However, beneath the place where the tail-bud forms or is already present in its first rudiment, there is a sometimes rather wide, sometimes small opening through which the nutrient yolk can still be seen from the outside. Where the tail is missing the blastopore lies exactly at the rear end of the embryo, has developed to the same degree as the tail, and is shifted ventrally and forward.

1. Group of Malformations: Total Blastopore Cleavage
(Table 16, Fig. 1-7) Page One Title

On the fifth to the seventh day after fertilization I noted among the embryos which had already developed considerably, individual eggs which lengthened in one direction and were pressed somewhat flat from the animal to the vegetative pole. Observed from the surface, these eggs to some extent presented the appearance of an oval dish which is colored throughout on one side by brown pigment, but appears cream-colored on the other side (Plate 16, Fig. 1-7). The brown side frequently shows a clear longitudinal inward curvature (Fig. 3 and 4); further it is seldom completely smooth, but more frequently is covered in an irregular manner with twisted furrows several places and, situated between them, with bulges which are comparable to cerebral convolutions. These are pathological proliferations of the ectoderm which are without any further significance for the development processes. Aside from this, an organ rudiment can frequently be found on the forward end of the brown side which the frog embryo forms only some time after the start of the gastrulation process. It is the brown adhesive disk (Fig. 3h) with which the hatched tadpoles attach themselves to water plants. The adhesive disk is an indentation of the epidermis surrounded by epithelial bulges which are roughly somewhat comparable to a horse shoe in shape. Thus, the brown side presents itself as the ventral side of the future embryo and its head and tail end can already be distinguished by the position of the adhesive disk.

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Therefore, the non-pigmented side corresponds to the dorsum which in our malformation presents a peculiar appearance. The yolk mass (d), which is divided into large cells, lies exposed (Fig. 1, 2, 4-7) and forms a nodule projecting now more, now less to the outside (Fig. 4-5d). This nodule is surrounded on all sides by a brown pigmented elevation (ur) which is formed by the spreading of the identically colored ventral side, to the dorsal side. The bulge is contrasted against the brighter yolk mass by a rather sharp line. Here too, the head and tail ends can be distinguished with some practice. On the former, at a small distance from the brown marginal bulge, a deep indentation exists which represents a transverse fissure (Fig. 1, 2, 6, 7 kd). As the cross-sections will show later on, it corresponds to a part of the gastrula invagination. The field situated between it and the marginal bulge also still shows a tinge of brown pigmentation. The posterior end, however, is distinguished by the brown marginal elevation being thickened into two humps (Fig. 1-7 sk) which are separated from the yolk mass by a deep indentation. The two humps, which I shall already designate as tail buds according to their future destiny, are separated from one another in the median plane by an indentation (Fig. 1 ar), in between which a small cell mass enters, that can be labeled an intermediate part and frequently causes a small prominence on the posterior end. Especially in later stages, the intermediate part can be observed more clearly (Fig. 9-11, 13, 14, 19), it has a groove (ar) which is related to the development of the anus and can therefore be labeled as the anal groove.

Nothing more can be learned by investigation of the embryo as a whole. Upon preparation of cross-sections, sagittal and frontal sections, one is all the more surprised to find a high degree of inner organization -- medullary folds, chord, middle germinal layer, primitive segments and so forth.

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In our discussion we best start with a cross-section through the center of the malformed embryo (Plate 17, Fig. 2). This shows immediately that all three germ layers have already been formed. The yolk mass which protrudes outward as a hump and represents the inner germ layer, is covered ventrally by two cell layers that are separated by a cleavage space, 1) by a thin outer germ layer, which possesses the known structure of the anurae and is most darkly pigmented, and 2) by a considerably thicker, somewhat less pigmented, middle germ layer (mk) which consists of several cell layers. Toward the dorsum of the malformation the outer and middle germ layer terminate in the brown colored bulge mentioned above which surrounds the yolk knob-like a ring when examined from the surface (Plate 16, Fig. 6 ur).

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In the cross-section, the bulge (Plate 16, Fig. 6 ur) shows a complex structure (Plate 17, Fig. 2). It consists of a thicker plate (mp), which is curved together to form a shallow groove that opens upward. In its structure it corresponds to the medullary folds of a normal frog embryo in that it is built up of several elongated, spindle-shaped cells which are stratified one upon another. Accordingly, it represents half of a normal spinal cord rudiment or half a medullary plate (see also Plate 17, Fig. 3, 6, 7, 10-12, 18, 22 mp). On the outer edge the medullary plate, by turning over, transforms into the thin epidermis; toward the inner edge it becomes likewise thinner and changes its histologic character, in that the cells become somewhat larger and less pigmented (Fig. 2, 3, 10, 12, 18, 22 v). Through this thin connecting piece (v) it is continuously connected with the yolk mass.

Second, the bulge includes on each side the section of a round cellular cord which is sharply outlined on all sides and corresponds in its structure to an embryonic chord. Because, as longitudinal sections through the latter show (Fig. 20 and 21 ch) it is built up of the known, disk-shaped cells which are stratified one upon another like coins. The chord is located somewhat medially from the bright medullary plate, exactly beneath the connecting piece (v), which constitutes the transition to the yolk mass.

The middle germ layer (mk) finally ensues beneath the chord and medullary plate. It is widest here and gradually narrows ventrally.

During examination of a series of cross-sections the individual pictures change only slightly. Toward the front the chord generally becomes somewhat stronger; and conversely somewhat weaker toward the rear. At the tail end finally, the medullary plate, chord, and middle germ layer can no longer be distinguished. They disappear here into a more homogeneous mass of small, pigmented cells which produce a bulge on either side on the posterior end

which, during examination of the embryo from the surface (Plate 16 sk), was already described as the tail bud.

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The surface relations between the marginal bulge and yolk frequently differ somewhat depending on the particular region of the body, but in a manner which does not always remain constant for the individual embryos. Sometimes the yolk is raised as a steep hump high above the marginal bulge (Fig. 2, 6, 11, 22); sometimes it terminates at the same level (Fig. 10, 18), or even shows a trough-like depression on the anterior and posterior ends above which, inversely, the marginal elevation now rises somewhat further (Fig. 3).

With all malformations that were sectioned, the middle germ layer already reveals an organization into primitive segments. Their number appeared to amount to 10 to 12 pairs, depending on the more or less advanced state of development. They can best be distinguished on sagittal sections which have cut the chord or the medullary plate, as in the sagittal section through a marginal bulge of the malformation of Ba. (Plate 16, Fig. 5; Plate 17, Fig. 20). Beneath the chord which is built up of disk-shaped cells (Plate 17, 20 ch) lie four primitive segments (us) sharply separated from one another which have been constricted from the remaining portion of the middle germ layer or from the lateral lamina. Five primitive segments (us) are visible in the sagittal section (Plate 12, Fig. 19) which cuts through the half medullary plate (mp) of embryo J³ (Plate 16, Fig. 7).

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It now remains to examine the condition at the head and tail ends of the embryo more closely. This is best done with sagittal sections. With the sectioned embryos the pictures turn out somewhat different.

In embryo Ea (Plate 16, Fig. 5; Plate 17, Fig. 8) a still completely open cerebral plate (mp) has developed in the head region. It is curved together to form a deep groove whose front or outer edge transforms into the thin horny layer while the inner edge changes into the yolk mass. A chord is lacking in the median region but appears laterally at some distance therefrom. This may be seen on the cross-section (Plate 17, Fig. 11 ch) and on the completely laterally directed sagittal section (Fig. 20 ch). In the embryo under study any invagination is lacking which might represent the first rudiment of a cephalic duct cavity. However, such an invagination confronts us in embryo J³, even if only slightly delineated (Plate 16, Fig. 7 Kd and Plate 17, Fig. 15). Behind the curved-in, thickened cerebral plate (mp) ensues a small hollow space (kd), which has formed by invagination. The orifice of this hollow space could already be observed in the study of the entire embryo. Consequently, the inner edge of the cerebral plate does not change directly into the yolk mass as above, but into a single layer of large yolk cells which define the front wall of the cephalic duct cavity and at their base double over into the compact yolk mass which constitutes the rear wall. The cephalic duct cavity is developed significantly further in embryos M and B (Plate 16, Fig. 10 and 2). As shown by the sagittal sections (Plate 17, Fig. 9 and 16 kd), it now extends downward not only to the base of the yolk mass in the region of the adhesive disk(h) but in

embryo M (Plate 17, Fig. 9) has also forced forward a protrusion beneath the cerebral plate (mp).

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In the investigation of the posterior end of the embryo we never miss a sometimes more, sometimes less indentation which may be considered as the rudiment of the pelvic intestinal cavity or the hind-gut (Plate 16, Fig. 1, 6, 7 ed and Plate 17, Fig. 8, 9, 15, 16 ed). It separates the yolk mass from both humps [labeled as tail buds (sk)] with which the marginal bulge terminates posteriorly and between which the intermediary cell mass with the anal groove (ar) is inserted in the medial plane (Plate 16, Fig. 1 ar). Each tail bud (Plate 17, 8, 9, 15, 16 sk) partially exhibits three layers, corresponding to the three germ layers. 1) toward the outside the black pigmented ectoderm, 2) toward the inside one or several layers of large yolk cells which form the back wall of the pelvic intestinal cavity, 3) between both, a thick layer of small, also black pigmented cells, or the middle germ layer. At the end of the tail bud a demarcation of the three layers from one another is lacking over some distance. They merge together in a mass of small cells into which a small furrow frequently extends (for the indentation, see Plate 17, Fig. 9 and 15 between the lip of the blastopore (ul) and the intestinal fold (dl)).

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Before turning to the description of more developed embryos it appears advisable first to deal more closely with the question which every reader will already have posed himself: how did this abnormal, peculiar embryonic form develop, and what is the relation of its development to the normal development of amphibians and vertebrates? Although, to my regret, I have never been in a position to find earlier stages of development of these malformations among my stock, I still believe that some fairly reliable conclusions may be drawn from the findings reported.

Without doubt, a completely disturbed gastrulation process took place. The abnormal development must already have been noticeable from the blastula stage on. Normally, an invagination develops from a small region which gradually becomes deeper in the place, which we, along with Götte, label as the marginal zone, and in which the animal, pigmented cells change into the larger, non-pigmented yolk cells. Following the little sac which developed first, an ever larger portion of the vegetative half of the blastoderm or the yolk mass gradually comes to lie inside of the double tube. During this process the blastopore constricts in corresponding degree to form a small hole, even before the medullary folds are formed; then it closes completely. In any event, this process must here have been altered completely and replaced by a different mode which may, however, also be described as a form of gastrulation.

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I feel that first the blastoderm lengthens somewhat in one direction and flattens somewhat from the dorsal surface to the ventral surface. Consequently the blastula cavity will disappear completely or partially. Then, on the future ventral side of the egg, and about the compact yolk mass, the animal cells form a covering layer which constitutes the outer germ layer.

Simultaneously the marginal zone or the place of transition of the animal and the vegetative cells will begin proliferating, at first on the forward end of the egg, then progressing from here toward the rear over its full extent. It will be transformed into a sort of germinal ring, from which primarily all developmental processes begin. On the left and right side of the oval egg, cell masses grow downward from the germinal ring between the yolk and outer germinal layer and produce the middle germ layer. If we visualize this process as folding in, then we obtain a result represented in the diagram in Plate 20, Fig. 17. By folding in at the marginal zone a little sac developed whose visceral germ layer is connected with the yolk mass while the parietal germ layer is transformed into the outer germ layer. I shall continue to label the outer transition margin as the blastopore lip. An important organ rudiment forms on it. Here, the ectoderm cells grow considerably in length and combine to a somewhat curved-in medullary plate (mp), which however, is equivalent to only half of a normal spinal cord rudiment.

In the further course of development the small sac closes along the lip of the blastopore (Plate 20, Fig. 18). In this way the inner margin of the medullary plate comes directly into connection with the yolk mass through an intermediate cell mass (v). Subsequently the small sac with its parietal and visceral layer detaches itself from the medullary plate and the yolk mass. In so doing the chord appears as a new organ from the band of cells which is labeled eh in Fig. 17. Thus the state of organization existing in our malformations, is brought about along the marginal bulge by interaction of fusion and separation processes. If one compares the processes, as I have presupposed them here, with the normal processes of development of a frog's egg, as they have been expressed in the schematic figures 19 and 20 in Plate 20, then one will easily find that they are essentially the same and also lead to essentially the same results. One must only disregard differences caused by the location. Processes which take place in a normal manner near the median plane of the dorsum take place here in the side region of the embryo at the place of transition of the animal into the vegetative half of the blastula, along Gotte's marginal zone or along the germinal ring.

A quite obvious displacement in the temporal series of the individual development processes is most intimately connected with the misplacement of the regions in which the nervous system, chord and primitive segment are first rudimentally developed. Normally the blastoderm of amphibians is first completely transformed into a gastrula; it becomes double layered over its entire surface. The blastopore is constricted to a very small slit. The medullary plate forms as a uniform, undivided rudiment in a region of smooth surface at a considerable distance from the remainder of the blastopore only after the gastrulation process is concluded. In all of our malformations, on the other hand, the gastrulation process has stopped in its first beginnings. Only the ventral and lateral wall of the embryo is covered by the outer germ layer while the yolk mass or the inner germ layer

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is exposed over the entire dorsal surface. In spite of this retardation of the gastrulation process, the higher organ differentiation which is characteristic of vertebrates (formation of the chord and spinal cord) sets in, thereby causing the pronounced local and temporal displacements.

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The embryos described are thus essentially arrest-malformations produced by disorder and arrest of the gastrulation process which has enjoyed neither a punctual beginning nor its normal conclusion. But therefore, they are due very singular and special interest. For among the numerous arrest-malformations known in teratology, that observed here appears to occur earliest in development. It concerns one of the most primitive conditions of organization not only of vertebrates but in general of the entire animal kingdom, namely the formation of the blastopore. No wonder, therefore, that the disorder thus produced has touched the fundamental synthesis of the entire body.

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A first glance at the cross-section through our malformation may easily lead some readers to a concept varying from mine. The eggs might be considered as double malformations in which each individual has reached only half its primordium. However, upon closer examination, such a concept proves to be untenable. Because, first of all the lateral primordia, which impress one at first glance as half-embryos, change into one another continually at the head and tail ends; second, they may later combine to form a rather normally shaped embryo, as the following section will show; third, the highest degree of arrest-malformation that is primarily discussed here is connected with normally developing embryos through all possible intermediary stages, which represent a lesser degree of arrest; and fourth, the adhesive disk for the entire egg is laid down singly and not doubly as would surely be expected in duplication.

Some interesting conclusions may be drawn from the statement that our malformations are gastrula forms which in one respect have undergone an arrest of their normal process of development in the process of invagination and in the closure of the blastopore, but in another respect have nevertheless progressed and already formed the most important organ rudiments of the vertebrate body. To this end let us reduce the malformation to a simplified scheme by visualizing the yolk mass in Fig. 17 (Plate 20) as consumed and replaced by a simple layer of intestinal gland cells. In so doing we obtain a gastrula form in which the still wide open blastopore, which is somewhat lengthened from the head toward the tail end, is enclosed all around by the rudiment of the nervous system. The lips of the blastopore themselves form a somewhat curved nerve plate, which lies free externally, i.e., a medullary ring.

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Such primitive form of nervous system is indeed permanently found in some lower divisions of the animal kingdom. It belongs to the highest organized polyps, the Actiniae in which they were discovered by my brother

and myself [26]. Such condition of the central nervous system is obviously extremely practical, because most central, since the different layers of the body, its outer and its inner surfaces, are in extensive and direct connection only at the blastopore. Therefore, sensory nerve branches originating at the outer margin of the medullary plate can go from here directly to the sense organs by spreading out through the dermal layer. Conversely, nerve fibers which originate at the inner margin of the medullary plate reach the muscle masses which develop from the cell material invaginated into the inside of the gastrula, from the inner germ layer (Actiniae), or from the middle germ layer (three-layered animals). The peripheral nervous system is thus completely and naturally divided according to its local origin into a sensory portion, emanating from the outer margin of the nerve ring, and a motor portion, emanating from its inner margin. Because the central nervous system among the vertebrates also comes to be established as a ring in the vicinity of the blastopore, as our malformations show so clearly, a very simple morphological explanation is offered for Bell's theorem or the fact of the separate origin of the sensory and motor root fibers, an explanation which I have already briefly discussed [25]. The former developed in conjunction with the outer germ layer from the outer or dorsal margin of a spinal cord half; the latter in conjunction with the muscle segments from the inner or ventral margin. Sensory and motor nerves need only follow the path already previously indicated in the natural stratified order of the germ layers in order to arrive directly at their end organs, the sensory fibers at the sensory organs situated in the ectoderm, the motor fibers at the primitive muscle bundles arising from the mesoderm.

The relations of the central nervous system of vertebrates to the blastopore (primitive furrow, blastopore) have already been frequently discussed and recognized. In this connection one point remained unclear, namely the extent to which the blastopore has split the central nervous system along its length. This problem is clarified by the arrest-transformations under study. The blastopore split initially divided the entire central nervous system into two equal halves which are closed at their ends to form a ring. Like the dorsal commissure of the cerebrospinal medulla, the ventral commissure is also formed only secondarily by coalescence. I shall return again in more detail in some closing observations to the question of the nature and manner in which this coalescence occurs in individual cases among the vertebrates after we have become familiar also with later stages of malformation.

My comparison of the central nervous system of vertebrates in its original stage of development with the ring-shaped nerve plate surrounding the mouth of Actiniae, or generally speaking, of Anthozoa, are in many respects in harmony with the phylogenetic speculations of A. Sedgwick [66] and earlier by Lameere [35] concerning the origin of vertebrates. "All the coelomateae descend in a way comparable to a scyphocnidare of the Actimozidae group whose mesenteric (positions) will be detached from the axial portion of the digestive cavity." Lameere bases this relationship on the ring

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shape of the nervous system surrounding the blastopore and on the formation of septa in Actiniae, namely in Cerianthus, which he takes to be a precursor of the body cavity divided into primitive segments. As further similar characteristics may be added the origin of the musculature and of the genital products, which in last instance are derived from epithelial cells of the inner germinal layer, because the middle germinal layer differentiates itself therefrom.

In stating these actual conditions, I agree with Sedgwick and Lameere but would prefer not to use these for phylogenetic conclusions which because of their nature can only be hypotheses of very dubious value. I can only repeat here the viewpoint which I have stated in the monograph on the Chaetognatha: "With all these analogies, I intend in no way to make probable a closer relationship of the animal divisions compared. For there are certain fundamental laws in the development of the organism according to which the establishment of tissues and organs ensues, from which proof of a closer relationship among different animals must not be immediately concluded, just as one could not conclude this from the uniformity of the process of cell division." The simpler the relations become, the more do points of comparison point in quite different directions. From the basic shape of a cup all metazoa can be derived, which still does not justify the conclusion that vertebrates are descended from the hydra.

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I see the scientific gain resulting from comparison of the stages of development of higher animals with developed lower organisms, and from the discovery of analogies between the two, primarily in the fact that we recognize states of form, which appear in the development of higher animals only as rudiments but in lower animals as functioning entities, and therefore learn to better appreciate their significance. The significance of the rod cells which we call the chord in the development of a bird or mammal becomes understandable only when we see the chord as a histologically differentiated structure actually functioning as a skeletal axis in a developed cyclostome. Equally, the results obtained with Actiniae make clear how, at a lower stage, a nervous system may develop and function around the blastopore.

II. Group of Malformations: Partial Blastopore Cleavage in the Dorsal Region

As emphasized already, the forms with partial blastopore cleavage present such numerous variations, disorders of a now more severe, now milder degree, that I divide them best into two subgroups in order to be able to give a clear presentation.

1. Malformations with partial blastopore cleavage in the dorsal region at an early stage of development (Plate 16, Fig. 9, 11-16, 18, 19)

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The forms of this group follow directly those previously discussed, insofar as the largest portion of the blastopore is still open and permits the yolk to protrude outwards; but they can be distinguished from the former

by the fact that they are generally somewhat further developed which is evident from the greater number of differentiated primitive segments. The fact that in these malformations a small portion of the head region has formed in an almost normal fashion is added as the second and most important distinguishing characteristic.

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The differences just emphasized are not as striking when the malformations are observed as a whole, only when a series of sections are closely examined do they fully reveal themselves. In all of them an adhesive disk has developed on the ventral surface of the head end, as in a portion of the malformations discussed earlier (Plate 16, Fig. 16 and 18 h). The presence of a small section of head can be recognized in the dorsal and lateral view by the fact that the exposed yolk is not hemmed in toward the front by a narrow, darkly pigmented bulge, but that a larger, pigmented field is present here (Plate 16, Fig. 6, 9, 12, 18, 19 k). Generally a round opening or a transverse fissure leading into the cephalic duct cavity (kd) is still visible directly behind the latter on the surface of the yolk knob. The lateral bulges (ur) though which border the yolk field in the middle show no change compared to those in the first group of eggs investigated, and often are equally far from each other. But the tail end has changed more considerably, it is also the portion which can vary the most in its appearance.

In Figures 11, 12, 13 (Plate 17), the tail buds (sk) have enlarged somewhat compared with earlier figures (Fig. 1, 2, 7 sk), and are separated by a deep indentation. At the end of the latter that leads to the ventral side, the anal groove discussed earlier is still more distinctly developed. (Fig. 9, 11, 13, 14 ar). It is bordered by two small folds (af) which turn back into one another toward the ventral side, similar to the primitive folds in the vicinity of the primitive groove.

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In Figures 18, 19, 15, 16 finally, the tail buds have grown into long processes (s). They never coincide in their direction with the long axis of the body but generally are bent over beneath the right corner toward the dorsal surface, which is best recognized by lateral observation of the embryo. Now and then the bending is even more severe and may reach such degree that the end of the tail processes runs parallel to the long axis of the body, lies across the yolk and is directed with the tip toward the head (Fig. 21 and 23). During growing up the tail processes may in rare cases remain separated down to the base where the anal groove lies (Fig. 16 to 19). Usually, however, they begin to fuse with one another from the base of origin so that only the tips remain as the last sign of the original duplication. The place of suture is clearly visible in Figures 11 and 14, and is labeled by the letter n.

Cross-sections made in a different direction will now provide us with a closer insight into the finer structure of the head region, of the central portion still bearing the blastopore cleavage, and of the tail end.

Longitudinal sections were made through embryo O (Plate 16, Fig. 12 and

11). Of these, Fig. 4 (Plate 17) represents a section situated near the medial plane. A cerebral canal (mr) segmented into several vesicles is contained within the closed head portion. Beneath it, with the exception of its forward third, lies a chord (ch) which tapers forward but is thicker than normal toward the back. Nerve tube and chord impinge upon the exposed yolk mass posteriorly and, in longitudinal section terminate with a sharp contour. If one constructs the bodily picture from the series of sections then one is convinced that in the places marked with a cross both organs split into two halves in order to surround the yolk mass in an arc on both sides. Shortly before the division of the nerve tube an auditory vesicle has been constricted right and left from the epidermis so that we can establish the place where the head ceases as a closed structure, in the vicinity of the medulla oblongata. The lower half of the head is occupied by the cephalic duct cavity (kd) which spreads into several sinuses and is covered by a high cylindrical epithelium. It opens outward through a fine fissure which rises dorsally through the yolk mass and leads directly between the head end to the little hole already noticed in the surface view (Plate 16, Fig. 12; Plate 17, Fig. 4 (kd)). The fissure separates a thin layer from the yolk mass. This layer adjoins the posterior end of the chord and nerve tube.

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A supplement to the sagittal sections is furnished by cross-sections which were made through embryo N (Plate 16, Fig. 9). The most anterior section of the brain to the vicinity of the mid-brain is normally developed. Thus Fig. 1 (Plate 18) shows a section which cuts somewhat obliquely through the mid-brain. The protrusion of the optic vesicle (ab) is found on the right side; whereas only the missing spot of the vesicle is found on the left while the remaining portion of the vesicle can be found on sections situated further forward. The floor of the mid-brain has bulged to form a cone (t) and adjoins the rather spacious cephalic duct cavity (kd). Beneath this ensues the middle germ layer which shows a small cephalic cavity to the right and left. The thickenings on the ventral surface of the ectoderm originate with the posterior end of the adhesive disk (Plate 17, Fig. 1 and 2 h). Finally, the auditory vesicle has been constricted from the epidermis (Fig. 1 hb). Because of the oblique direction of the section this vesicle can only be found on the left while the right follows only on a later section of the series (Fig. 2 hb).

At the same time Fig. 2 gives an idea of the division of the cerebral furrow which has been produced as a consequence of the blastopore remaining open. From a central unpaired section of the cerebral furrow, which probably corresponds to the region of the mid-brain, two lateral vesicles (mr) bow out. These probably contain the formative material of the medulla oblongata. To the left the hollow space and to the right the forward wall of the protrusion are found. The covering of the brain, which is thus separated into three lobes, is represented only by a very thin epithelial layer, to which the epidermis is directly attached as a very fine membrane. Only the lateral walls of both outpocketings show a considerable thickness. Beneath the left the forward tapering end of a chord (ch) can be seen, which here

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follows a rather oblique course. On the other side, a few sections later, a similar chord can also be perceived beneath the other protrusion. In embryo N, therefore, the portion of the chord lodged beneath the brain has been laid down in two completely separated halves.

In later sections the central portion of the brain rudiment disappears. Right and left protrusions separate from one another and each forms, in cross-section, a tube which is thickened laterally and composed of long, spindle-shaped cells which are shoved into one another, while medially the tube is closed only by a thin layer of flattened cells. The cross-section of a chord adjoins each tube. In the series of sections we now come to the region where the head end, that as a whole is normally formed, ceases completely and where the yolk mass pushes through the blastopore, which has remained open, between the central organs which have split and separated, i.e., between the left and right halves of the nerve tube and chord, etc. A section situated somewhat in the middle of this segment is illustrated in Fig. 3 and a small part of it, the region of the lip of the blastopore, is illustrated at a still higher magnification in Fig. 4.

To the left and right of the yolk which protrudes outward as a hump, both halves of the spinal cord (mr) are found. Unlike the embryos of the first group studied earlier (Plate 17, Fig. 2, 3, mp), they no longer represent exposed plates, but have closed to form a tube. As in the normal closure of the spinal cord the medullary fold has risen more above the surface on each side and curved inwards toward the median plane, until along with the marginal fold, it has met the juncture of the medullary plate with the yolk, designated in Fig. 2, Plate 17 with the letter v, and has grown together with it. The tube formed in this manner (Fig. 4 mr) corresponds to only half of a spinal cord rudiment. For only its outer wall shows the cellular composition of a nerve plate; the inner wall consists of a thin layer of flat cells which medially adjoin directly the large yolk cells. In spite of the prevented closure of the blastopore, the central nervous system has thus separated from its parent tissue. It is now situated on the lip of the blastopore, covered externally and above by a thin epidermis, and with its inner surface adjoins the yolk which replaces the intestinal gland membrane, and rests below upon the chord which constitutes a round, altogether sharply circumscribed bundle. In many sections, between spinal cord and epidermis there is a small knot of cells (Fig. 4 g) which appears to have formed by proliferation of the lowermost cell layer of the latter; it is the rudiment of a spinal ganglion. From outside and below the chord is enclosed by the primitive segment (us) which is sharply delineated from the remaining portion of the middle germ layer, the lateral lamina.

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In order to complete our understanding of the conditions of the head end in this second group of malformations, I shall briefly go into the results shown by the cross-sections through embryos Bg, F and X.

In embryo Bg (Plate 16, Fig. 18 and 19), a small piece of a normal cerebral furrow is enclosed only in the most anterior end of the head; toward the back (Plate 18, Fig. 16) it quickly expands in width, whereby only the

lateral walls (mr) remain thickened, whereas the floor and the covering thin out to an extremely fine epithelial membrane (vp). In the middle, a chord (ch) appears in the medial plane between the halves which have separated. The chord is exceedingly strongly developed. To the right it extends a lateral branch which pushes beneath the corresponding cerebral hemisphere, while a smaller hump appears to the left. Upon close examination of the series of sections, the picture clarifies in that the chord forms unpaired and assumes the correct position between brain and cephalic duct (kd) only at the beginning, but then splits into two separating branches, of which the right is more conspicuously developed. Accordingly, in the following sections the middle piece of the chord is seen disappearing together with both epithelial layers which, in Fig. 16 vp, extend between both cerebral hemispheres. Simultaneously, the cephalic duct cavity opens to the outside on its upper wall. This finally produces the cross-sectional picture shown in Fig. 17 (Plate 18).

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The middle of Fig. 17 is occupied by the yolk mass which is divided into large cells and cut from top to bottom by a cleavage-shaped hollow space (kd). Toward the front it is connected to the cephalic duct cavity, and toward the top it opens onto the surface of the exposed yolk in the depression that was previously noted during examination of the surface (Plate 16, Fig. 19 kd). The upper margin of the yolk or the entrance into the primary intestinal cavity, which still remains open, is limited on both sides by the separated halves of the medullary tube and by the chord. These have developed in a different manner on the right and left. To the left, a thick medullary plate is attached to the yolk by a narrow connecting piece. The surface of the medullary plate is covered by the horn blade for some distance. Beneath the junction of medullary plate and the yolk, the cross-section of a very retarded chord rudiment (ch²) can be seen. But to the right the rudiment of half the spinal cord has closed to form a tube (mr) in the manner previously mentioned for embryo N. The horn blade continues across the tube and attaches to the yolk. The chord (ch) finally, on this side, is developed as a conspicuous bundle.

In embryo F (Plate 16, Fig. 13), the blastopore has become closed not only in the region of the head, where the auditory vesicles have already separated from the horn blade, but also in an adjacent portion of the back. The cross-sections shown in Figures 18-21 (Plate 18) were made through the transition of the closed portion into the clefted portion of the blastopore. In the first of these, the spinal cord is already widened and its covering transformed into a thin epithelial layer. From this, a thin septum (Fig. 18 sch) extends toward the base plate of the spinal cord. This septum is found on several sections of the series and consists of flattened pigmented epithelial cells. It separates the central canal in a left and right part. The unpaired chord (ch) pushes between spinal cord and intestine, which is filled with large yolk cells and shows only a vertical, narrow split (kd). The chord is also widened in transverse direction. In one of the following sections (Fig. 19 ch), this widening has increased even more. The lateral halves of the spinal cord, in whose wide central canal the septum has again

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disappeared, are also slightly more separated. Further toward the rear (Fig. 20) the chord has separated into a strong right (ch) and a very inconspicuous left bundle (ch). The further widened spinal cord has been opened to the outside by the disappearance of the thin covering. Its lower surface is fused with the upper wall of the intestine for a short distance between the separated halves of the chord (Fig. 20). Finally in Fig. 21, which is one of the next cross-sections, the separation of all organs is complete. Between both halves of the spinal cord the base plate has completely disappeared so that only the yolk mass of the intestine enters directly into the void and connects with the left and right medullary plates through an intermediary part. In the process the above-mentioned cleavage-shaped void has broken through the upper intestinal wall in the yolk, so that in this embryo, as in all preceding cases the cephalic duct cavity has been provided with a direct opening to the dorsum between the lips of the blastopore.

The often unequal development of the organ halves on both lips of the blastopore becomes evident on review of the series of sections through the dorsum of different embryos. From the completely symmetrical behavior shown in embryos J and N, for example (Plate 17, Fig. 2 and 3; Plate 18, Fig. 3 and 4), there are transitions (Plate 18, Fig. 17 and 20) to cases where there is no organ rudiment whatever on one side of the blastopore.

Embryo X (Plate 16, Fig. 15 and 16) presents such extreme case where the organ differentiation has already made great progress on the inside. The head is nearly normally developed only in the most anterior section and is equipped with adhesive disk (h) and auditory vesicles. Then, the blastopore cleavage begins already at the dorsum and is wide open and filled with exposed yolk mass.

As the investigation of the entire embryo in incident light has already shown, the lip of the blastopore (us) has developed on the left side (Fig. 16) to form a thick fold spreading toward the rear into a long tail end (s) which separates from the yolk mass and is then dorsally bent at right angles. Examined in cross-section, the fold (Plate 18, Fig. 27) is shown to consist of 1) half a spinal cord rudiment (mr) which has closed in the familiar manner to form a tube and is covered by the horn blade which is attached to the yolk; 2) a strong chord (ch); 3) of a primitive segment whose cells are already at the point of differentiating to form primitive muscle bundles.

On the opposite side (Plate 16, Fig. 15) the lip of the blastopore (ur) is barely indicated by a brown pigmented band which separates the dorsal surface which consists of yolk cells, from the ventral surface which is covered with pigmented cells. Here and there the band is raised as a small fold a little above the surface, especially toward the tail end, where it is transformed into the boundary of the anal groove (ar). On the cross-sections we may then reliably verify that on the right-hand lip of the

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the blastopore all organ development is suppressed (Plate 18, Fig. 27 ur). No trace is present of a spinal cord rudiment, a chord, or even of a middle germ layer. On the entire right side the yolk mass is covered only by a thin layer of ectoderm which is attached to the yolk cells at the location of the blastopore lip and is raised thereby in several sections to form a more or less widely projecting fold. The right tail bud is lacking. On the head end the cerebral vesicle on the right side is also formed only in a thin epithelial layer.

The cross-sections through the middle of the trunk of embryos F and Bg show the transitions which lead to this extreme case of unilateral stuntedness.

In embryo F (Plate 16, Fig. 13; Plate 18, Fig. 20 and 21) the right chord (ch) is strongly, the left (ch^l) slightly developed; in the middle of the trunk it even disappears completely for some distance so that the otherwise well developed medullary plate impinges directly upon the primitive segments. In the tail region (Fig. 22, 23) it appears again although it is still stunted in relation to the other side. In another embryo I was also able to observe such a partial chord development, a stunted rudiment in particular sections, and a complete lack in others. Last, embryo Bg (Plate 16, Fig. 19) is of interest because in it the defective rudiment extends also to the spinal cord in addition to the chord (Plate 18, Fig. 17). On the right side it has closed to form a tube (mr), on the left side it remains as open plate, and this also lags behind the other side as regards the mass of its substance.

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Of special interest for several morphological problems is accurate study of the tail end, which has already provided such striking results deviating from the normal condition during surface examination of the malformations.

In two embryos, Bg and X (Plate 16, Fig. 18, 19 and Fig. 15 and 16). both tail buds (S) remained separated, in spite of the fact that they had grown significantly in length. In general, they consist of a mass of small cells, yet a cross-section through its base shows that organ differentiation has already begun even here (Plate 18, Fig. 26). The ectoderm has clearly developed as a separate layer except in one spot on the dorsum where it is thickened and changes into the inner cell mass, the middle germ layer. Close to this spot, the cross-section through the rudiment of the spinal cord (mr) and the chord (ch) is located in the left tail bud. Both are very inconspicuous. The spinal cord is closed to a small tube whose wall is thickened only laterally and medially consists of a layer of cells which are pressed flat. On one side it is bordered by ectoderm, on the other side by the middle germ layer, in which the formation of the primitive segment has probably already begun if other embryos are any indication; this however, could only have been established by longitudinal sections. The chord is situated medially, ventrally from the spinal cord. The right tail bud appears smaller because it is cut somewhat further away from the base. Here, only a small spinal cord canal (mr) of the familiar composition can be distinguished. The chord rudiment, however, disappears in the small

celled tissue which is separate everywhere from the ectoderm. A small hump, the intermediate part (z), is encountered between the two tail buds. On its surface, the anal groove, which is so clearly developed in most malformations, makes its way. In the more distal sections all organ differentiation ceases or is at least no longer recognizable, so that every tail bud consists now only of small, pigmented, thickly crowded mesodermal cells, which are covered by a thin layer of ectoderm.

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In the second embryo X (Plate 16, Fig. 15, 16), in which the differentiation of organs has already made considerable progress, only the left tail rudiment is developed, whereas the right is completely stunted like the entire right lip of the blastopore.

In cross-section it is shown as being composed of a rather conspicuous sharply defined chord, of half a spinal cord rudiment which has closed to form a tube, and of primitive segments which have already differentiated muscle fibers.

I consider the knowledge that the blastopore changes its character in one small region at the posterior end of the embryo as the most important result of the findings reported in the preceding pages. While it otherwise differentiates everywhere to form the spinal cord rudiment and the chord, the first with its ectodermal layer, the latter with its endodermal layer, this process of differentiation ceases in the region which becomes the anal groove. The portion which differentiates into the above organs may be designated the germinal ring. It is thus posteriorly interrupted by the anal groove. At this interruption its two ends develop to form tail buds which grow beyond the anal groove and differentiate further in the same manner as the halves of the germinal rings into the individual rudiments, spinal cord, chord, and middle germ layer.

The embryos Bg and X, which were just examined for the nature of their tail end, form exceptions among the majority of malformations. For in most cases both tail buds begin to grow together at their medial surfaces as soon as they attain larger dimensions and become more prominent above the surface. Depending on whether this process of fusion is initiated earlier or later, and further depending on whether it is completely or only partially finished quite different appearances are produced. Exact analysis of this process is of great morphological interest because it clarifies the development of the anus, a question which has been repeatedly debated during the past years without being brought to a really satisfactory conclusion. Considering the diversity mentioned above and the importance of the subject, I report a larger number of findings furnished by the series of sections through embryos J (Plate 16, Fig. 6), N (Fig. 9), O (Fig. 11, 12), G (Fig. 14), and F (Fig. 13).

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In embryo J' (Plate 16, Fig. 6) the tail buds are firmly joined in the medial plane and have thus produced an undivided transverse fold which closes the blastopore cleavage posteriorly. Somewhat further back of this fold the anal groove is visible which in front begins at some distance from

the blastopore cleavage, then deepens further and is bordered by clearly developed anal folds, which turn back into one another ventrally. Because the fold has pushed in between, the anal furrow does no longer form a caudal protrusion of the latter which is connected to the blastopore cleavage as in embryos J³, Bm, M, and Ba^{ver}. Rather, it is separated from the anal furrow as an independent structure. Fig. 13 in Plate 17 shows a cross-section through the point of coalescence, and Fig. 14 a section through the anal furrow. In Fig. 13 the point of coalescence is denoted 1) by the arrangement of cells; 2) by the darker pigmentation, so that a black streak passes dorsoventrally through the mass of small cells; 3) by a contraction of the surface in the region where the ectoderm cannot be distinguished from the lower cells for some distance. Beneath the fused tail buds a small intestinal space is delineated. This pelvic space is the intestinal cavity, whose wall consists ventrally of numerous, large yolk cells which are stacked in layers one upon another. As direct continuation of the germinal ring, the tail bud still shows some organ differentiation on the left side. The chord (ch) is delineated in the mass of small cells as a band of cells running parallel to the yolk surface. Therefore, the mass of small cells above probably represents the medullary plate; and below probably the middle germ layer. Toward the point of coalescence all of the germ layers grade into one another in a fashion similar to what happens in the regions of the primitive streak in vertebrates. /387

A number of sections further to the rear (Fig. 14) the pelvic intestinal cavity has disappeared except for an oblique split which is bordered by yolk cells. The ectoderm is thickened dorsally and in the medial plane shows a cone shaped indentation which is surrounded by two folds of the ectoderm protruding above the surface. In this location the ectoderm cells have become somewhat tall and almost cylindrical. This picture reappears in a rather similar fashion in quite a number of sections. In the region of the anal groove a place is now especially distinguished by the fact that in this spot the ectoderm cells directly adjoin the yolk cells which line the hindmost end of the pelvic intestinal cavity. In this spot the pelvic intestinal cavity will later open through the anus to the outside. Except for this spot the anal groove otherwise borders everywhere on the middle germ layer.

The cross-sections through the tail end of embryo N (Plate 16, Fig. 9) present a complete pendant where the tail buds appear dorsally as somewhat heavier folds and have combined in the medial plane so that they now delineate the open blastopore toward the rear and have separated the anal groove from it as a separate part.

The cross-section (Plate 18, Fig. 5) passes directly behind the portion of the blastopore that has remained open and through the forward beginning of the place of coalescence. On the right side the organs are still well separated. These can be followed inward from the germ ring for some distance until they disappear from view in an undifferentiated mass of small cells. The half rudiment of the spinal cord (mr) is cut somewhat obliquely because it changes here from a straight to an oblique course. Beneath it we find

the chord (ch), limited toward the outside by the middle germ layer, and by yolk cells toward the inside; as in embryo J, these line a closed, small pelvic intestinal cavity (ed). To the left side the tail bud is much less developed and composed only of three cell layers, the ectoderm, a middle germ layer of especially small cells, and the yolk cells which line the pelvic intestinal cavity. At the place of coalescence and somewhat beneath it, all three germ layers again grade into one another on the left side. Some sections further to the rear (Fig. 6) the hollow space of the pelvic intestinal cavity ends while the yolk cells (ed) which terminate its rear wall are still encountered. The latter form a central mass which is completely surrounded by the middle germ layer and is clearly separated from it ventrally, while dorsally both grade into one another. As in the primitive furrow of chickens, here too, the outer germ layer cannot be separated from the middle layer in one groove-like deepened spot.

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In one of the next sections (Plate 18, Fig. 7) we find ourselves in the region of the anal groove (ar), in which the ectoderm is raised in two folds (af) and the ectoderm cells are distinguished through size and cylindrical shape. Ectoderm and middle germ layer no longer grade into one another. On the contrary, the latter is again absent for a short distance, in that the yolk cells (ik) of the pelvic intestinal cavity extend up to the anal cavity and are laid closely against the ectoderm cells. To the left and right of the process formed by yolk cells we note the small cells of the middle germ layer, which however, are not delineated from the latter by a sharp split. Still further to the rear the anal groove extends for some distance but abuts everywhere against the middle germ layer from which it is clearly separated.

A medial section through embryo O (Plate 16, Fig. 11 and 12) and two series of frontal sections through embryos W and G will provide further insight into the complicated phenomena occurring at the tail end during development of the anus.

The sagittal section through embryo O (Plate 17, Fig. 5) departs a little from the medial plane in an oblique direction. This may easily be recognized from the fact that not only the region of the anal groove is cut, but that the free end of a tail bud is also encountered.

The latter should not be visible in a section falling exactly in the medial plane because the ends of both tail buds are still separated by a gap and are fused over about half their length only. Therefore, the gap should have been in the region of the section. The tail bud which bends a little over the rear surface of the yolk, consists of a mass of very small cells, in which organ rudiments cannot be distinguished because only the ectoderm is deposited as a separate layer. In the region of the anal groove, the outer germ layer is considerably thickened and here shows a small indentation from which a connection exists to a rear and downward protrusion of the pelvic intestinal cavity. The outer germ layer changes here clearly to an upper and lower lip and continues into an epithelial

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layer that lines the rear wall of the pelvic intestinal cavity. On the lower lip the middle germ layer has grown downwards, separating yolk and ectoderm from one another. If we imagine the tail bud in the figure removed, then the picture is completely equal to the medial sections through embryos Ba, M, J⁵ and Bf (Plate 17, Fig. 8, 9, 15, 16; Plate 16, Fig. 5, 10, 7, 2), where the tail buds have hardly developed yet and where the intermediate piece with the anal groove are completely exposed. Accordingly, we should visualize the condition in Fig. 5 (Plate 17) as resulting from an earlier condition represented by embryos Ba and M such that when the tail buds rise above the rear surface of the embryo by considerable growth, they move together with their basal portions at the anal groove and from here begin to coalesce toward the periphery. Only in one small place in the region of the anal groove does fusion cease, and a direct connection is retained here of the ectoderm with the segment of the pelvic intestinal cavity that is situated farthest to the rear and ventrally. This connection represents the anus.

Both series of frontal sections through embryos G (Plate 16, Fig. 14) and W, both of which represent milder degrees of arrested blastopore closure, show a similar picture. In both, the tail buds have already joined very prematurely by a suture (n) in the medial plane over their full extent, before they appeared free above the surface. They form a wide transverse fold which encircles the exposed yolk mass toward the rear. As a result, the anal groove (ar) is pushed away from the yolk mass and can best be seen if the embryo is placed on its head and the hind end turned directly toward the observer. The groove, which is surrounded by low folds (af), no longer runs horizontally but vertically from top to bottom, so that frontal sections are better suited to study them. Forward of the anal groove a fine line can be traced up to the yolk knob. This line is produced by a slight contraction of the surface. In a way it represents a sort of seam, a suture, along which the fusion of both tail buds has taken place (Plate 16, Fig. 14 n).

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Frontal sections of embryo G are presented through three different regions (Plate 18, Fig. 10-12), one section through the suture at some distance above the anal groove, a second section through the forward end of it, and a third through its hind end.

In the first section (Fig. 10) the fold produced by fusion of the tail buds is chiefly composed of thickly crowded, small, pigmented cells of the middle germ layer; the ectoderm is clearly separated up to the region of the suture where it is fused with the mass of small cells at the base of the groove. Toward the primitive digestive tract which pushes between yolk and tail fold as a narrow fissure, this mass of small cells is covered by an entoderm layer which can be distinguished as a separate layer only at some distance from the medial plane. On the left side of the section, the mass of small cells of the tail bud transforms toward the front into the region of the lateral lip of the blastopore, where the chord and medullary plate have started to develop. A piece of the chord (ch) is directly cut by the section,

In the second section through the middle of the anal groove, which is drawn at somewhat larger magnification (Fig. 11), the individual rudiments are changed somewhat. The ectoderm has thickened and formed two folds (af), which contain between them a small cavity which is lined by cylindrical cells. The ectoderm is followed by the middle germ layer which consists of many layers of small, pigmented cells. However, it is interrupted in the region of the anal cavity; a process emanating from the entoderm extends to the base of the latter. This process is not separated by a fissure from the fissures of the middle germ layer, but can nevertheless be distinguished by the considerable size and the low pigment content of its cells. The entoderm layer, which together with this process extends up to the anal cavity, is 2-3 cell layers thick and forms the rear wall of a very fine fissure which terminates the pelvic intestinal cavity in this region (ed).

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In the third section through the rear end of the anal groove (Plate 18, Fig. 12) the picture changes once more. Both anal folds (af) lie close to one another, separated only by a very narrow groove (ar). Beneath the ectoderm follows the middle germ layer as a continuous layer, well defined by fissures toward the inside and outside. Then comes the yolk mass with the extension of the pelvic intestinal cavity (ed), which disappears in the next sections.

Frontal sections through the hind end of embryo W (Plate 18, Fig. 8 and 9) yield a pendant. Fig. 10 of embryo G (Plate 18) corresponds to Fig. 8, which is a section through the coalescence suture, where outer and inner germ layers have fused with the middle germ layer in the medial plane. Fig. 11 of embryo G can be compared with Fig. 9 of embryo W. Here, even more than in the former case, the anal groove (ar) stands in wider, direct connection with the large celled entoderm (ik), which closes the ventral outpocketing (ed) of the primitive intestinal tract toward the rear. Here too, there occurs a completely sharp separation of the middle germ layer from the point of connection between anal groove and the rear intestinal wall. To that extent the picture presents a state of development somewhat further differentiated than that reproduced in Fig. 11.

In my paper concerning the middle germ layer of amphibians [24] I have already given a corresponding illustration of the anal groove of a normally developed frog embryo. We are here concerned with a stage of anal development which I have since been able to observe repeatedly in a series of sections through normal frog embryos.

Last, embryo F (Plate 16, Fig. 13) should be noted, whose two tail buds (sk) have already developed to greater length and have fused to considerable extent. Since their long axis describes almost a right angle with the main axis, the sections were cut in frontal direction. This produced the series of cross-sections from which the following four figures were drawn (Plate 18, Fig. 22-25).

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In Fig. 22 the beginning of the tail is cut behind the yolk knob. The

surface turned to the back is deeply hollowed out like a furrow. Both margins of the wide furrow are occupied by the two spinal cord rudiments (mp) which, running around the exposed yolk knob [where they were described by me in the above cross-sections (Plate 18, Fig. 21)], have again approached the medial plane; however, they are still separated by a band of yolk cells which defines the rear wall of the hind gut (ed) and continues to the beginning of the tail bud. There, where the medullary plates thin medially and attach directly to the yolk layer, we encounter among them again the cross-sections of a left and right chord (ch) even though it is very inconspicuous. Laterally the medullary plates are transformed with a reflected margin, which is raised above the surface like a fold, into the ectoderm which covers the convexly arched ventral surface of the embryo. Beneath the medullary plate and chord there lies on each side a band of small, pigmented cells, which make up the middle germ layer and are later divided into the primitive segments of the tail. Both bands are separated by a wedge of yolk cells pushing between them. These yolk cells represent the intestinal gland layer and toward the rear enclose a small cavity as an outpocketing of the hind gut.

In one of the following sections (Fig. 23) a closure has formed on the dorsum so that the yolk mass is no longer exposed. The lateral cover folds of the medullary folds have grown (vp) to make contact toward the medial plane and have united with one another. Thus a very flat, band-shaped spinal cord (mr) has been formed which easily allows recognition of the fusion of the two halves which were still separated a short time before, because only the lateral portions are thickened and are united in the middle by two rather broad, thin epithelial membranes (vp). Two separate dorsal cords (ch) are also found beneath the band in the former position. In the cross-section through the tail we further note both bands of the middle germ layer and between them a band of yolk cells (inner germ layer) with a very small cavity (ed) which ventrally directly adjoins the ectoderm.

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The third section (Fig. 24) follows soon after the one just described and shows a mass of small cells, only slightly differentiated, in which only the following rudiments can be distinguished indistinctly: 1) the two mesoblast gands (mk) and the yolk bands (ik) separating them, which dorsally grade into one another for some distance; and 2) two bands which form the extension of the medullary plates. Chord rudiments can no longer be recognized. The hollow space in the yolk band has also disappeared. The entire small-celled tissue is completely enclosed by a clearly separated ectoderm. Ventrally this has formed two widely projecting folds (af) which, on a whole series of sections, define the anal groove (ar) that has already been described with the surface of the embryo. The base of the anal groove directly joins the yolk cells (ik) and is attached to the beginning of the groove directly at the point where the small cavity (Fig. 23 ed) was contained in the yolk band so that an opening of the anal groove would again produce a direct connection with the protrusion of the hind gut.

The cross-section (Fig. 25), finally was cut near the tip of the tail

end. A separate ectoderm layer encloses a mass of small cells which no longer shows any differentiation into individual rudiments. From the dorsal surface, which still shows a small furrow, a black pigment band grows vertically through the germinal tissue toward the ventral surface, connecting both ectoderm surfaces, which here are thickened somewhat and not very well delineated from the cells situated inside. Thus, the pigment band still indicates the coalescence surface of the two tail buds in the same manner as the pigment band illustrated in Fig. 8.

2. Malformations With Partial Blastopore Cleavage in the Dorsal Region at a More Advanced Stage of Development Embryos A, B, C, P, S, T, V
(Plate 16, Fig. 17, 20-23)

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In the second group I classify malformed embryos which have developed considerably further than the forms discussed in the preceding chapter. For these, a longer interval (2 to 3 days more) has already passed since fertilization. At the head the eye rudiment can be externally perceived because a small lens sack has already formed and the eye vesicle is invaginated to form a cup. In some embryos (Plate 16, Fig. 20, 22) external gill plumes already begin to sprout forth. The tail reaches a greater length and is sometimes completely enclosed by a small fin edge (Fig. 20-22 sf). In body and tail the primitive segments have developed in great number, and in the body have begun to differentiate into muscle fibers. The mesonephric ducts are established in full length, and are provided with a wide cavity. The primordium of the heart is present.

Upon external examination of the embryos only little can still be noticed of the great derangement which must also have existed in them as an extensive blastopore cleavage at an earlier age. The principal characteristic from which an earlier derangement may be inferred is the peculiar position of the tail with respect to the body. As is the case in normal embryos, the tail does not lie in direct extension of the body axis but is strongly curved back at its root toward the dorsum. Either it forms a right angle to the long axis, as already observed in the first primordium of the tail bud in the first group, or it is even more strongly curved upward and forward, so that the tail end finally runs parallel to the dorsal surface and lies close above it. Thus the embryo is in a way bent along its dorsal surface to form a ring. Embryos S, V and C (Plate 16, Fig. 20 and 22) show the milder degree of pathological arrangement of the tail; embryos A, B, and P (Fig. 21 and 23) show the highest degree.

If one now examines more closely such characteristic malformations of the degree of development specified, on the dorsal surface in front of the attachment of the recurved tail one will find now a greater, now only a small fissure, into which a portion of the yolk passes. This dorsal fissure had mostly disappeared in embryos A, B, (Fig. 21 d) and C; whereas in embryos S, V, P, the fissure had disappeared except for a small hole (uo), through which only a little of the yolk could be seen (Fig. 20, 22, 23 uo). Further, at the root of the curved tail end and at its convex side which

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corresponds to the ventral surface, a small groove can be seen hemmed in by skin folds. The groove often extends for some distance into a furrow that continues toward the tail end (Fig. 20-22 a and ar). The entire groove looks not unlike a medullary groove in miniature, so that at the beginning of my investigation, the supposition could emerge that the rudiment of a second, very rudimentarily deposited, accessory embryo might be present on the main embryo. As closer examination will show, we are again dealing with the structure already described as the anal groove. This becomes best visible when the embryo is artificially placed so that it turns the hind end directly toward the observer. Upon lateral inspection one notices in some larvae that the tail root is separated by an indentation from the ventral surface of the body and that the anal groove begins with a small swelling (Fig. 20-22).

Among the more developed, older malformations there was also one which was still equipped with a continuous tail fissure (Plate 16, Fig. 17). The hind end ended in two long processes (rs and ls) which were curved toward the back and originated on both sides of the well developed anal rudiment (ar). With their cone-shaped end they were crossed over each other so that the left half tail side reached out toward the right across the medial plane and the right half reached across toward the left.

More precise information about the degree of malformation which exists in the embryos of this group can be learned only from cross-sections made in either transverse or frontal direction. Figures 12-14 (Plate 19), which are taken from a series of cross-sections through embryo A, give information on the condition of the forward and middle portion of the body. In its exterior, this embryo is so much like embryo B illustrated in Fig. 21 (Plate 16) as to be mistaken for it, so that I have given no special illustration thereof.

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The first cross-section through embryo A is located at some distance behind the auditory vesicles (Plate 16, Fig. 12). The spinal cord (mr) with heavily thickened lateral halves is somewhat stretched in width; the chord (ch) situated below it, which is now composed of vesicle-shaped cells filled with fluid, is no longer circular as in the preceding sections but is changed into a cylindrical body from left to right. Beneath the chord follows the esophagus (d) which conceals a small cavity; beneath this the cardiac tube (hz) which contains a number of blood corpuscles and is enclosed in the pericardium.

In the following sections both lateral halves of the spinal cord separate still further and are held together dorsally and ventrally only by a thin cell membrane. Thus the situation represented in Fig. 13 is developed where the doubling of the spinal cord and chord that was already indicated by broadening, is now complete. The spinal cord has disintegrated into two tubes which in the medial plane are separated by a small interspace filled with homogeneous colloid matter. Each tube is composed of a lateral, heavily thickened wall and a medial wall which is only a fine membrane

consisting of flattened cells. Above both, the ectoderm extends as continuous cover (vp) along the dorsal surface. Beneath each half of the spinal cord lies a chord (ch¹ and ch²) of oval cross-section formed by a fork-like division of the forward unpaired chord section. The intestinal tube pushes from below between both halves of the chord and in part between both neural tubes, crowding the divided organs apart. Thus the way is paved for the condition shown in Fig. 14.

In its entire appearance Fig. 14 is generally like the cross-section through the corresponding region of malformation N (Plate 16, Fig. 9; Plate 18, Fig. 3 and 4), which is younger by several days. Only now all organ rudiments have differentiated considerably farther and, therefore, also cause a thicker fold on both sides of the central yolk mass which projects as a hump; the following parts of the hump can be distinguished in the figure: 1) the half rudiment of the spinal cord (mr) which is closed by a membrane to form a tube; 2) a spinal ganglion, which grows downward between the lateral thick wall of the spinal cord and the ectoderm; 3) the large chord (ch) which is circular in cross-section and which is enclosed by the primitive segment (us) laterally and ventrally in a half-moon shape; 4) the mesonephric duct (ug) with a small cavity which pushes in between the primitive segment and lateral lamina. In the latter the body cavity becomes here and there visible as a fissure. The ectoderm discontinues above the spinal cord tube with a thickened fold which is laterally attached to the yolk mass.

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Sections cut through the embryo in horizontal direction permit even faster orientation about the character and degree of malformation than cross-sections. For this purpose, the embryo which was curved together dorsally to form a half ring, was in most cases oriented so that the most dorsally situated point of the head and tail coincided with the plane of the section. Therefore, in a large part of the sections the embryonic body was cut twice (head and tail, each by itself), i.e. these portions yielded more or less clear cross-section pictures.

I refer next to the three horizontal sections through embryo V (Plate 16, Fig. 22). On its back in front of the tail root only a small stomate (uo) is still present through which the yolk, however, no longer protrudes to the outside. On the contrary, at the opening the epidermis sinks toward the inside like a funnel. Correspondingly one notices in all three horizontal sections (Plate 19, Fig. 7-9) a hole (t) defined by irregular contours. This hole is widest in the section (Fig. 8) made mostly dorsally, while it is severely narrowed in Fig. 9, which represents a section of the series made more ventrally. The epithelial membrane (ak) that borders the hole consists of black pigmented epithelial cells.

On the middle of the three sections (Fig. 7) the spinal cord (mr, mr¹) is cut almost exactly lengthwise. It forms an oval ring surrounding the blastopore cleavage, which toward the tail end is interrupted for a short distance. The left and right halves of the nerve ring represent a tube whose lateral wall is heavily thickened in the manner familiar from the cross-sections, while the medial wall is closed only by a fine epithelial

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membrane. This clings directly to the epidermis which sinks deeply in a funnel-shape to form the border of the blastopore cleavage (t). Where the thickened lateral walls of both halves of the spinal cord meet in front and combine to form an undivided rudiment, we discover the section of the chord (ch) which is of rather considerable size here. It is cut somewhat obliquely, because it turns back from the horizontal into a more vertical direction in order to penetrate into the head end of the embryo, which is curved upward. Also toward the tail end two chord sections (ch) can still be seen. They lie directly beneath the halves of the spinal cord which end freely toward the rear. They are very inconspicuous and separated from one another by only a narrow band of yolk substance (ds). Both cross-sections are easily explained in this fashion: Beneath the separate halves of the spinal cord two chordae dorsalis also make their way in horizontal direction and, having reached the rear end they turn at right angles in order to penetrate into the vertically rising tail end.

Laterally from the nerve ring and from the multiple chord sections lie numerous primitive segments (us) which have already differentiated into muscle fibers toward the front and are sharply separated from each other by black pigmented lines. Their number is different on both sides, to the left 12, to the right 16, probably because in the rear portion the section does not pass through corresponding segments of the middle germ layer.

Toward the front, the foremost part of the body is cut which then continues dorsally into the head segment. Here, the largest mass of yolk is accumulated in the intestinal canal which, as a consequence, is greatly swollen. Further, one finds here the cross-section of the conspicuous mesonephric ducts (ug), located on each side between the primitive segment and lateral lamina.

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If we pursue the series of sections somewhat further toward the abdominal region (Fig. 9), the nerve ring disappears gradually; instead the horizontal segments of the chorda dorsalis rise beneath it. The latter begins in front with a greatly thickened head, and toward the rear divides into two branches. Each branch tapers toward the rear and then turns at right angles into the tail end, so that two cross-sections (ch) were found in the picture described previously (Fig. 7). In Fig. 9 both branches of the chord directly include a portion of the intestinal canal, which is filled with yolk cells. This corresponds to the cross-sections obtained from other embryos, where both halves of the chord with their medial surfaces cling always more or less directly to the yolk in the region of the blastopore cleavage (Plate 17, Fig. 2, 3, 7, 18, 22; Plate 18, Fig. 3, 4, 21; Plate 19, Fig. 14). Moreover, one notices even in the middle of the fork, which is formed by the splitting of the chord, the end of the ectoderm indentation (t) which opens in this region with a small opening into the intestine. Since it narrows in a funnel shape, it requires a much smaller space than in Fig. 7. Sections made still further ventrally beneath the chord lead completely into the intestinal space, which is filled with yolk mass. The latter forms the continuation of the nutrient yolk which is shown in the foremost portion of Fig. 7; on the basis of analogy with embryos Bg, F, B

(Plate 16, Fig. 18, 13,21) it probably was exposed during an earlier stage of development of embryo V to great extent on the dorsum at a time when the blastopore was still wider open.

If we now pursue the series of sections dorsally in opposite direction from that chosen as a starting point for the discussion and presented in Fig. 7, the nerve ring soon disappears as continuous structure (Fig. 8). Toward the front it continues in the foremost, normally developed segment of the spinal cord tube (mr), which turns in an oblique direction into the head and is therefore not cut precisely across in this figure. In front of it is the extraordinarily well developed chord (ch). Toward the rear we encounter the two cross-sections of the portion of the spinal cord (mr) which is split into two halves which turn back here into the root of the tail in the form of two small tubes situated close to one another, together with the halves of the chord that belongs to them and is also visible in cross-section. Between the three cross-sections of the undivided and divided spinal cord the section cuts the outer opening of the funnel-shaped blastopore cleavage on the surface of the skin.

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In the following sections of the series, the central connection between the head and the tail segment of the embryo disappears. For we reach the region where these parts rise freely above the curved-in dorsal surface. Thus we obtain partly oblique sections, partly pure cross-sections of these which will occupy us later.

Corresponding series of frontal sections were made from embryos S and P (Plate 16, Fig. 20 and 23). The frontal section through embryo S (Plate 19, Fig. 22) as a whole is very much like that in Figure 7. The spinal cord is cut in the spot where, divided into two halves like a fork, it encloses the blastopore cleavage (t). The latter is even wider here than in Fig. 7; both halves of the spinal cord are somewhat farther from one another. In the present frontal section the blastopore cleavage is enclosed toward the front by ectoderm which, as in embryo V, sinks toward the inside for some distance and downward grades into the yolk mass. To the right the tube-shaped halves of the spinal cord together with their thin-walled epithelial closure plate, adjoin the sunken ectoderm. Toward the rear, the border of the blastopore cleavage is formed by the yolk mass, which can also be seen for a short distance from outside; and which from here continues into the tail root. The chord is again cut three times as in Fig. 7, partly across, partly oblique; a more presentable oblique section is found in front of the place where both halves of the spinal cord (mr) have united to form a normal, unpaired rudiment; two further smaller chord cross-sections (ch^1 , ch^2) lie at the rear end of each spinal cord half and, like the latter, can be followed rather far into the tail rudiment.

If we continue ventrally in the series of frontal sections, we soon chance the point of forking of the chord beneath the spinal cord division. Meanwhile in Fig. 23, which furnishes an illustration of such a section, only the right portion of the branch is encountered, while the left follows

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in one of the next sections. At the point of forking, the chord is thickened to form a fold (ch^2) which causes a protrusion into the primitive intestinal cavity. Such tumor-like proliferations of the chord (chord tumors) are not at all rare in older malformations in the region of the forward body segment, especially in front of and at the point of division. In some cases I have observed them in even greater extent than in the preparation shown here.

Finally, in Fig. 15 (Plate 19) a section is illustrated which has been cut through embryo P (Plate 16, Fig. 23) in a more or less transverse direction; thus it directly cuts the entrance into the blastopore cleavage, from which some yolk still appears, but not in exactly horizontal direction so that left and right halves present a somewhat different appearance. On the right side the section exposed the one spinal cord half which limits the blastopore cleavage in its entire length. At its forward and rear ends a chord cross-section is always found, a larger one (ch) forward, a smaller one (ch^1) behind. Both belong to the right chord half that is situated in the blastopore region, continues in horizontal direction beneath the longitudinally cut spinal cord and then turns in front and back at right angles in order to rise vertically into the head and tail of the embryo, which is curved together to form a half ring.

On the left side of the figure the section has only grazed the surface of the most deeply inflexed point of the back. Therefore, on the left of the blastopore one sees only the epidermis, that limits it evenly cut and making the connection between the cross-section of the tail root situated to the rear and the cross-section of the body situated in front. One notices the spinal cord (mr) and chord (ch^2) twice in the cross-section, first in the forward segment of the embryo, secondly in the tail root. The connecting piece belonging to each organ, which runs along the left lip of the blastopore in horizontal direction, first appears in subsequent deeper sections. The blastopore cleavage is enclosed by ectoderm (ak) toward the tail, by yolk mass toward the front. The transversely cut tubes (ug) are the mesonephric ducts. They cling to the primitive segments which envelop the chord unilaterally.

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On the three embryos, V, S, P the still preserved remainder of the blastopore cleavage occupied the dorsal region in front of the tail root. However, it may also happen that the remainder of the blastopore cleavage falls in the middle of the region of the tail root itself. Actually, I have observed this interesting condition only in one single embryo, namely embryo T (Plate 16, Fig. 17), but here with all desirable clarity in a complete cross-section series. Even upon surface observation of the entire embryo (Plate 16, Fig. 17) a total splitting of the tail could be presumed. Both halves (rs and ls) of the tail rose at right angles above the dorsum curved like a hook and their ends were crossed over one another. The investigation of the cross-section series (Plate 19, Fig. 21) showed further that both halves of the tail were separated from one another to a complete extent

only some two-thirds of their length distally; but that in the region of the tail root, i.e. at the place of transition into the body, a partial fusion had occurred not only dorsally but also ventrally. I say partially because only the epidermis of the dorsal and ventral sides was affected by the fusion. On the other hand a narrow fissure extended through the middle of the tail root and through it one reached directly from the outside into the pelvic intestinal cavity. This fissure was present in six consecutive sections, from which one is illustrated in Fig. 21. One can pursue the right curved tail half (s^1) from the root up to the hook-like, turned tip; and in longitudinal section one notices a thin chord (ch) and above it a not very clearly separated spinal cord half, and on the opposite side of the chord numerous primitive segments (us) clearly separated from each other. Of the left tail half only the root (s^2) and its tip (s) is encountered, while the connecting piece, which is crossed by the tail end of the other side, shows up only in lower sections. In this half, which to me appears to be retarded in its differentiation, the chord and spinal cord cannot be recognized separately from the small-celled tissue, while primitive segment formation is noticeable but not as clear as on the opposite side. The above mentioned fissure now passes between both tail roots and in it the ectoderm changes into the yolk mass and through it one gets into the intestinal space.

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It remains to concern ourselves still more closely with the peculiarities of the tail end and with the anal groove in the embryos of this group.

In most cases the tail appears externally to be normally formed, if we disregard its peculiar arrangement that has already been emphasized (Plate 16, Fig. 20-22). While in earlier stages the tail develops from two lateral halves and shows a partial doubling, even if it has already grown to rather considerable size, as in the case of the embryos illustrated in Plate 16, Fig. 12, 13, 8, 18, 19) no doubling can be observed externally in this group, with the exception of embryo J (Plate 16, Fig. 17), whose pronounced double tail was already described. Only the investigation of a series of cross-sections shows that nerve tube and chord have also doubled in these older embryos and that they present half rudiments, which still show evidence of the earlier presence of a blastopore cleavage.

The results which are presented vary somewhat, as shown by the cross-sections through different embryos in Figures 1-3, 10, 11, 16, 19, 20, 24 of Plate 19. Both spinal cord tubes and both chordae dorsalis lie farthest apart in the tail root since the remainder of the blastopore cleavage which still remains open ordinarily extends in their region. From there they gradually draw together toward the tip of the tail until they both touch and also fuse in some cases. The nerve tube becomes extraordinarily small and narrow, but at first still shows that only the lateral wall is thickened and consists of spindle-shaped, stratified cells, whereas the medial wall is a fine membrane of flat cells. Each tube is therefore only half a spinal cord. The chord cross-section is likewise very small, but is clearly

separated from the surrounding tissue. In all cases where the double rudiments lie apart, the interspace is filled with yolk cells which continue from the wall of the pelvic intestinal cavity into the tail as a narrow band and thus represent a kind of postanal gut which, of course, always lacks a hollow cavity.

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The septum, which is formed from yolk cells, also extends between both bands of small cells, which belong to the middle germ layer and from which the primitive segments of the tail differentiate. In the tail many primitive segments have already been formed on both sides and they can best be recognized in oblique and longitudinal sections. Toward the tip of the tail they disappear while a small celled germinal tissue appears in their place which is destined for subsequent further differentiation.

The rudiments of the spinal cord and chord lie farthest apart from one another in Figures 2 and 3 of Plate 19, which were cut transversely through the tail root of embryo B (Plate 16, Fig. 21). The yolk mass (Fig. 3 d) that protrudes from the blastopore cleavage which extends this far, contains a small fissure as the last offshoot of the pelvic intestinal cavity. In one of the ensuing sections (Fig. 2) this fissure has disappeared toward the tip of the tail; at the same time the septum (d) (postanal gut), which consists of yolk cells, has become more narrow in this section, so that the nerve tube (mr) and the chord of the left and right side have moved closer together. This has happened even more on the third section (Fig. 1), which passes through the tail end, which, in turn is once again curved forward in a hook shape, as Fig. 21 of Plate 16 shows. Since the body portion of embryo B had served to make a series of cross-sections, the extreme tip of the tail is lacking in Fig. 1 because it fell into the region of the other series of sections. Since this tail piece is cut more or less lengthwise because of its being curved back, one now sees numerous primitive segments (us); two nerve tubes (mr) running lengthwise, but separated only by a small interspace; and in the medial plane, the postanal gut (ds), a long band of yolk cells which separates the rudiments of the left and right sides.

In embryo S (Plate 16, Fig. 20) the double rudiments of the nerve tube and chord lie far apart in the tail root; in the middle of the tail (Plate 19, Fig. 24), which has already developed a conspicuous fin crest (sf) dorsally and ventrally, the double rudiments are still separated, to be sure, but have moved quite close together. Between the left and right chord (ch), but no longer between both nerve tubes (mr), yolk cells (ds) are present as dividers.

The condition of embryo P is similar (Plate 16, Fig. 23). Fig. 15 (Plate 19) represents a section through the tail root of embryo P, Fig. 16 a section through the middle of the tail. Here, a fin crest (sf) has only grown dorsally. The postanal gut (ds) pushes here not only between both chord halves, but also between the nerve tubes (mr) which are farther apart.

The series of sections through embryo V (Plate 16, Fig. 22) and C show further peculiarities. At the tail root of the former the double rudiments of the spinal cord and chord (Plate 19, Fig. 8 ch, mr) are already separated by a narrow septum of yolk cells (ds). In the cross-section through the middle of the tail (Fig. 11), which is again equipped with a dorsal and ventral fin crest (sf), both nerve tubes (mr) touch and are fused with one another through their thin medial walls in such a way that together they form a septum between the central canal which is still double. A band of yolk mass from the postanal gut (ds) still pushes in between left and right chords (ch¹, ch²). In other sections (Fig. 10) the septum has suddenly disappeared in the spinal cord (mr) so that only the cross-sectional appearance of the central canal that is shaped like the figure 8 still points to the original doubling.

Finally, in embryo C I came upon one last variation (Plate 19, Fig. 18-20). In the cross-sections through the middle of the tail the spinal cord is simply formed in normal manner; however, the chord doubles beneath it (ch¹, ch²). The chord halves, which have approached almost to the point of touching, are separated by no other tissue. Beneath them one discovers the roundish cross-section of the compact postanal gut (ds).

And now some words regarding the anal groove. In embryos of this stage in almost all cases it has developed quite clearly on the ventral side of the tail root and is expressed in a peculiar manner (Plate 16, Fig. 17, 20-23, a, ar). Namely, two segments can now be distinguished in it, a groove-like initial portion and a tube-like terminal segment which is either formed close to the wall of the pelvic intestine or has come into open communication with its cavity. For the most part the entire structure passes through a greater number of the sections of the series (Plate 19, Fig. 1, 2, 5, 6, 19, 20, 22, 23 ar). Like the tube, the furrow (ar) is lined by a single layer of tall epithelial cells filled with pigmented granules.

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I give a short description of anus formation based on some figures which are taken from the series of sections through embryos B, V, S, and C.

On the ventral side of the tail of embryo B (Plate 16, Fig. 21) the anal groove is hemmed in by very high folds (af) in several sections (Plate 19, Fig. 1). These folds form a clear protrusion on the surface. The picture is not unlike the cross-section through the medullary groove. If one takes into account that the groove then closes in the same manner to form a tube, as Fig. 2 ar shows, then, if one does not understand the relationship of all the phenomena, the assumption might easily be made that the spinal cord rudiments of a stunted, second embryo are present. That this supposition is erroneous follows, however, not only from the entire relationship, but primarily also from the fact that the tube, which opens to the outside as a groove, is connected on the inside with the intestine. Even in Fig. 2 the tube directly adjoins the yolk mass and in one of the following sections it has opened into the hollow space which now appears as an outpocketing of the pelvic intestinal cavity into the yolk mass (Fig. 3 d).

The position of the groove-shaped segment vs. the tube-shaped one is always such that the former is turned toward the tip of the tail, the latter toward the tail root, or in other words, the former lies distal, the latter proximal.

In embryo V (Plate 16, Fig. 22) the ventral tail reaches far downward. The anal rudiment, which here opens outward very briefly and only in one place (Plate 19, Fig. 5 ar), and then, closed to form a tube (Fig. 6a), joins the pelvic intestinal cavity, begins where the ventral tail ends.

Embryo S (Plate 16, Fig. 20) again shows us the entire anal rudiment developed to greater length and projecting far above the surface. At the lower end of the ventral tail fin the furrow opens which is hemmed in by extraordinarily high folds. In Fig. 22 (Plate 19) we see it, closed to form a tube (ar) and covered by ectoderm. In Fig. 23, a section of the series following even further ventrally, the cavities of the anal tube (ar) and the pelvic intestine (ed), which meet here, join into one.

Embryo C (Plate 19, Fig. 18-20) presented a divergent condition in so far as the anal rudiment and the pelvic intestinal wall remained here completely separated with a tissue layer lying between them. Fig. 20 shows the groove (ar), Fig. 19 the new growth of the groove to form a tube. However, it terminates blind, and is separated from the yolk (ds) by a stratum of middle germ layer (mk). Only several sections later, after the anal rudiment is no longer present, does a cavity appear in the yolk mass (Fig. 18 ed). Therefore, I feel that in this case a malformation known as atresia ani may have occurred as the result of special circumstances. It also appears to me, which is probably connected therewith, that the entire position of the anal tube is somewhat abnormal, in that it has moved up from the tail root more toward the tail end.

In addition to the second group of malformations I shall discuss two further embryos. Although they show disorders in the closure of their blastopore, these are of such slight degree that the subsequent development of spinal cord and chord is apparently not adversely affected (Plate 16, Fig. 26 and 27). Both embryos are still in a relatively early stage of development. The egg has changed from a spherical to an oval shape and is in the stage of the first rudiment of the medullary plate. In the embryo shown in Fig. 26 the two medullary folds (mw) appear in the normally developed dorsal region in their full length both still separated from one another by a wide distance and bordering a lyra-shaped field. The egg looks in every respect normal up to the point that the blastopore (uo) on the hind end, where the thinned medullary folds stop, still presents a rather wide and round hole and permits a large yolk plug to protrude from it to the outside. In entirely regular development the blastopore would have to have changed at this time at the hind end of the embryo into a quite small longitudinal fissure with lateral lips closing together.

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The second embryo (Fig. 27) has, on one hand, developed a little further than the one just discussed, because the medullary folds (mw) in the forward half of the dorsum already approach one another and together begin to combine. On the other hand, the disorder in closure of the blastopore is somewhat larger. For in the rear half of the dorsum the blastopore presents a very wide, round hole, from which a large hump of yolk mass

protrudes. The medullary folds, already preparing for closure in the forward region are consequently seen in the rear half of the dorsum turning at right angles toward both sides in an arc, whereby they become lower and are soon lost in the blastopore. A cross-section through the middle of the yolk plug (Plate 19, Fig. 27) therefore shows that even the medullary plate has not yet differentiated. At the lip of the blastopore, which forms a wide elevation enclosing the yolk, the outer and inner germ layer change without interruption into the middle germ layer (mk), which sinks into the depth between both as a somewhat pigmented band of small cells.

If we ask ourselves how, in both these cases, the further course of development might probably proceed, then I think that I can answer this without any hesitation in the first embryo, no disorder in the formation of chord and nerve tube will occur and it will presumably come to a delayed, to be sure, but otherwise regular closure of the blastopore and to a complete overgrowth by the medullary folds. In the second case, however, the absorption of the yolk mass into primitive intestine is still so far behind that the medullary plate and chord will differentiate on both sides at the lips of the blastopore even before its closure. In Fig. 27 both organs are already present on the left side in their rudiments. Therefore, an arrest malformation of a slight degree has occurred, with a spinal cord split and a chord split, somewhat of the type which we became acquainted with in Fig. 14 (Plate 16).

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That the blastopore remains excessively wide at a time when the medullary folds are already forming as in the embryos shown in Fig. 26 and 27, is moreover a phenomenon which can more frequently be observed in eggs which develop very slowly as a consequence of deficient oxygen supply. In the middle of spawn balls which are raised in small vessels without water change, one probably always finds such eggs in larger number, as I recall from other occasions. Up to now I have not yet examined objects retarded in this manner more closely. In the publication cited in the introduction, Roux also mentions that formal aberrations appear frequently, caused by retardations or inhibitions of some processes but are often adjusted in the further course of development.

III. Group of Malformations. Partial Blastopore Cleavage in the Anal Region.

In the third section embryos are discussed which are already relatively far and apparently normally developed, in that their entire dorsal region is separated into nerve tube, chord and primitive segments and optic vesicle, auditory vesicle and adhesive disk are established (Plate 16, Fig. 28-31). However, they all have a common characteristic which deviates from the normal; they possess a sometimes smaller, sometimes larger opening (uo), either on the hindmost end of their longitudinally stretched bodies if no tail has yet been formed, or, if one is already present, on the ventral side directly in front of the tail root. At the opening the white yolk mass protrudes from the darkly pigmented environment like the Ruskonian yolk plug.

From four embryos, which I selected for illustration and cut into a series of section, one shown in Fig. 28 has an immense, oval yolk field at the hind end. At the upper lip of the field the first rudiment of the tail (sk) is visible in the form of two humps.

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In embryo Ac (Fig. 29) the yolk field occupies a corresponding position but is only about a third as large; in embryo Ab (Fig. 30) it has moved on the ventral side more to the front and can therefore be seen only on ventral examination of the embryo. This situation is easily clarified from the fact that in the elongation of the back, the rudiment of the tail has grown out a little toward the rear over the yolk hole. Of these subsequent states embryo H (Fig. 31) is the last for it already possesses a tail projecting far to the rear and bordered by a small fin edge. In front of the ventral root of the tail, almost in the same position as in embryo Ab, embryo H possesses an oval opening (uo) filled by yolk mass. /410

In all of these cases we are also dealing with the remainder of a blastopore cleavage and in this regard this group naturally agrees with the malformations discussed in the first and second groups. Only here it is the portion of the blastopore situated farthest toward the rear and ventrally, which is retarded in its normal transformation and has been kept open in an abnormal manner. Frontal and sagittal sections provide still further explanations about these and other relationships.

In the cross-section through embryo Ad (Plate 16, Fig. 28 and Plate 19, Fig. 17), which still possesses the widest opening, it becomes noticeable that the outer germ layer is directly attached on the ventral side to the exposed yolk (ul²). While dorsal and lateral lips of the blastopore (ul¹) have been established on gastrulation, the formation of a ventral blastopore lip (ul²) has ceased. However, since the pelvic intestinal cavity is formed by this lip behind the yolk mass, this is also lacking in this case.

In the longitudinal section of embryo Ac (Plate 16, Fig. 29 and Plate 19, Fig. 26), whose blastopore has become much narrower, we encounter a ventral blastopore lip (ul²) but as a whole it is very insignificant. Certainly the entire yolk mass appears very severely crowded toward the rear.

In connection with the defective formation of the ventral lip of the blastopore all embryos in the third group further lack an anal groove and are thereby distinguished from the malformations comprising the first and second groups where the anal groove is a structure which appears comparably stronger than in normal embryos. Among these embryos (first and second groups) however, the ventral lip of the blastopore as well as the anal groove are not only very premature but also especially strongly developed and have formed a pelvic intestinal cavity. See especially Plate 17, Fig. 8, 9, 15 ed. /411

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We cannot designate the openings discussed here as ani, although they

correspond in position to them. As a distinguishing characteristic between the two I would like to consider one point as being decisive. At the lips of the blastopore, outer and inner germ layers do not stand in direct connection with one another but are separated by the middle germ layer, which originates here. At the anus, however, ectoderm and entoderm grade directly into one another while the middle layer is separated from both and possesses a large perforation corresponding to the anal opening.

The embryos of the third group discussed thus far present no abnormalities in the rudiment of their remaining organs. Embryo H (Plate 16, Fig. 31), which already possesses a small, special tail portion, constitutes the only exception upon examination of the series of cross-sections. Fig. 28, Plate 18 shows one of the cross-sections cut through the remainder of the blastopore (uo) and the beginning of the tail root. Ventrally one sees the yolk field, still not overgrown and enclosed by lateral, small blastopore lips. Dorsally, a trace of a dorsal fin can be noticed. The spinal cord situated beneath is outwardly simple, but shows a fine cell membrane (sch) in its relatively wide central canal. The membrane pushes in from above like a septum. Beneath the spinal cord lie the cross-sections of two very small chord bundles (ch), both separated by yolk cells, which push in between from the dorsal wall of the intestine. This doubling of the chord can be observed in a series of sections. It soon ceases, toward the front, while toward the rear it continues for some distance into the tail.

From the result at hand it may be safely concluded that the blastopore cleavage, which now exists only on the later anal position, did, at an earlier stage of development, extend dorsally up to the region where the tail buds are formed, i.e. still more toward the front than in the case of embryo Ad (Plate 16, Fig. 28). The blastopore cleavage must still have been present at the time when the first differentiation into nerve tube and chord took place in this region. During its closure which occurred soon afterward, the doubling of the chord remained as the last sign of disorder.

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Critical Examination and Evaluation of the Findings

In order to relate the findings concerning the peculiar malformations of frog eggs set forth in the previous pages, both to the older concepts of morphological systems and to the further improvement and expansion of morphological precepts, I would like to discuss in detail the following points in three chapters:

1. The occurrence, in other vertebrate animal classes, of relevant malformations, and their place in the system of teratology.

2. The importance of some of the findings from examination of malformations, in relation to such basic concepts of vertebrate animal morphology as:

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- a. the blastopore theory and the concrescence theory,
- b. tail development and secondary growth,
- c. the coelom theory.

3. The relationship of the blastopore lip to the formation of malformations and the possible causes of some of these malformations.

1. The Occurrence of Similar Malformations in Other Classes of Vertebrate Animals and Their Position in the System of Teratology.

The malformations of frog eggs observed by Roux and myself have no exceptional significance in the system of teratology. Various malformations have been reported in bony fishes by Lereboullet, Oellacher and Rauber, and have been designated by Oellacher as "terata mesodidyma." Studies of salamanders have been made by Klaussner and by Richter on chicks. Also in mammals and in man, malformations seem to occur which belong in this text and will be discussed under the category of "spina bifida".

a. The terata mesodidyma and katadidyma of bony fishes

The reports of Lereboullet, Oellacher and Rauber are of the greatest interest because they observe the origin of malformation in a relatively early state and their outcome for sometime thereafter. The fundamental observations of Lereboullet [38] are set forth in his well-known work: "Investigations on the Monstrosities of the Pike, Observed in the Egg and On their Mode of Production". By artificial fertilization of relatively small and transparent pike eggs the French researcher has produced a large proportion of offspring with two and three heads, also greatly dwarfed embryos without heads, and finally a fairly numerous group which produces anomalies which in every respect compare with our malformed frog embryos and which had aroused his earliest interest. These were embryos which had a simple head in the front and a simple tail in the rear but had two bodies in their center that were split in such a manner that they formed a more or less elliptical ring (Plate 16, Fig. 24 and 25). Both arms of the ring united at the front into a single head, and at the rear into the single tail. Each branch of the ring upon careful examination, as Lereboullet points out, is not a complete body, but only a lateral half. One can differentiate in each side a spinal half (mr) and a chordate half which unite in the head portion to a normal spinal cord and a normal chorda. Furthermore, one notices on either side a simple row of primitive segments which only encompass the outer side of one branch so that it appears as if they were the result of a lengthwise division of a simple embryo into two symmetrical halves. Also the simple head has only 2 eyes and 2 auditory vesicles. On the other hand, there was in a great number of cases a definite heart in each half, as if nature was attempting to duplicate the same organ in both halves.

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If the embryo lived for a time, both half-bodies came together and united at the mid-line to such an extent that finally only a slight ring-like opening at the base of the tail remained. Lereboullet therefore concludes, "Embryonic duplication arises here from the separation of the symmetrical parts of the normal embryo. That is what makes me say that the two embryonic bodies in these anomalies are in reality only half-bodies."

From this sort of malformation it becomes apparent that of the two body halves which enclose the opening into the yolk sac from the germinal disk one is either retarded in its development or is completely suppressed, a situation which, I have noted, is the same in the frog malformations (Plate 16, Fig. 15-16 and Plate 18, Fig. 27).

Lereboullet could observe in the pike also the first formation of the following malformation in the ring-forming germinal mound (embryogenic swelling). In anomalous eggs the latter develops only a short and thick cephalic process which does not elongate into a primitive streak; in this case the germinal mound itself became unusually thick to a complete extent (Plate 18, Fig. 30); "it was manifestly much richer in organizable material". The germinal mound dissociates forthwith into a row of single primitive vertebral plates in the same way as it would arrange itself along the medullary groove in a normal embryo. The two rows unite toward the front as the basis of the head protuberance and at the rear in the region which subsequently gives rise to the tail. "Thus, in summary, in the 'monstrosity' with which we are concerned, the embryonic swelling gives rise only to the cephalic region, but is itself transformed to form the embryonic body, and this body is composed of two halves because of the annular form of the generative swelling. The embryonic swelling should therefore be considered as a mass, a sort of storehouse of organizational elements, and as the point of departure of all the embryonic formations, regular or anomalous."

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I have gone somewhat extensively into the work of Lereboullet because it not only contains many worthwhile observations but also because these observations are made with the correct point of view. On the whole the work of Oellacher [42] "on terata mesodidyma of *Salmo salvelinus*" is of the same rank.

Oellacher carried out artificial fertilization of eggs of chars which he obtained from a 12 mile stretch of the Innsbruck Valley. Among the fertilized eggs, whose number he estimated as 400-500, he found an astonishing number of malformations which practically all belonged to the genus *Mesodidymi*. Their count exceeded from 10 to twenty times the number which he had previously carefully established in numerous fertilizations and observations of trout. In my opinion, this massive incidence of malformations in this instance is due to the long and certainly arduous transport from a mountain stream of the spawning female fish in which the ripe eggs were harmed resulting in fertilization by more than one spermatozoan during the subsequent artificial fertilization.

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The malformations examined by Oellacher are on the average older and

more widely illustrated than those described by Lereboullet. In them the cleavage is either very deep and extended for a long stretch (in extreme cases from the auditory vesicles all the way to the tail) or it is shallow and takes in only a short stretch of the body, or finally it is very short and makes only a slight indentation in the surface of the trunk.

The examinations of Oellacher provide, as I have stated, an enlargement of the work of Lereboullet particularly in as much as the malformations were also examined by transverse sectioning. In this way, it was clearly established that all paired organs are in no way affected. The eyes, hearing organs, primitive vertebral plates, primordial kidneys, pectoral and abdominal fins were never found doubled. /416

Doubling, or rather cleavage, could only be detected in unpaired organs originating in the median plane, most of all the spinal cord and chorda, outside of these but still in many cases the heart and intestinal canal and the liver descending from the latter.¹

In the doubling of the heart, intestine and liver, these fish malformations differ in an interesting way from the frog embryos wherein I never observed anything similar. I reproduce here, to illustrate the point, from the work of Oellacher a cross-section through an altered embryo of *Trutta fario* in the region of the liver rudiment (Plate 18, Fig. 29).

One can notice in each half-body a rolled up spinal cord half (mr), the chorda (ch), cells of which have already become alveolar, the already greatly enlarged primitive vertebral plates (us) the Wolffian duct (ug), underneath leaning up against the yolk is an intestine lined with a simple epithelial sheet (d), arising from this the liver rudiment (l) which consists of many convoluted tubules. From one to the other body-half, bridging the small interspace, not only the epidermis but also the ectoderm falls over, the latter as it lies directly upon the yolk.

The most apparent differences which are noticed between malformations of the frog and of the fish, particularly the doubling of heart and intestine, can be understood from the normal development of the heart and intestine, particularly between haloblastic and meroblastic eggs. In the latter the heart normally is in the form of two side by side tubes laying up against the large nutrient yolk during its development, while in the amphibian from the very beginning the heart is ventral in the egg and /417

¹ The observation of Oellacher that in the foremost part of the cleavage a median primitive vertebral plate occurs on occasion appears to me, especially in view of the foregoing findings of Lereboullet, to be due to an exchange which was perhaps induced when a cell mass from a lateral primitive vertebral plate pushed under the chorda to the median side.

unpaired. Even so, in meroblastic eggs the intestine separates from the entoderm as a recognizable tube by formation of a fold off the main mass which formed the yolk sac, while in amphibians intestine and yolk sac are never separate from each other.

In his transverse sections through fish malformations Oellacher determined that in the body parts which seem outwardly to be normal, in the fore body and the tail, that the doubling of the median organs progresses little further and only gradually progresses to the normal state. Here the two intestinal ducts unite first into a simple primordial structure, then the two spinal cord halves and finally the two strands of chorda as in the case with the frog embryo.

Finally, I note that Oellacher observed some even older Mesodidymi which hatched out of the egg membranes and which have already lost their yolk sac for one to two weeks earlier. "These outwardly are normal individuals and no one would take them for mesodidymi if he had not previously observed the characteristic curvature of these individuals in earlier stages, in stages when their inner duplication was still outwardly visible." This also reminds one of the similar condition in older frog embryos.

The parallel between malformed frog and fish embryos can be further extended. In the frog I have observed a malformation in which the forepart of the body was normal from its widest point forward while the cleavage occurred in the rear end and the entire tail so that the two tail buds had not fused. Oellacher has written of similar malformations in charrs under the title of Katadidymus. These are embryos, as he has remarked, "in which the tail bud and adjacent part of the rear body up to the upper germinal layer which forms a bridge between the two is completely split."

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A third investigator who has worked on specific cleavages of the body of bony fishes is Rauber, whose observations are set forth in his well known presentation "Formation and Disturbance of Formation in the Development of Vertebrates." Rauber [50, 52] has written about a goodly number of fairly young germinal discs of the trout and salmon in which a smaller forward part develops normally but the adjacent body part is split, being separated into two halves by a considerable interspace. Left and right halves of the embryonal ring have already differentiated organ beginnings without having come closer in the middle or having joined together in the normal embryonal form. Between the separated halves of the embryo is the yolk sac which is not exposed but is, as Oellacher has previously stated, covered with a thin cell sheet which belongs to the ectoderm and which spans from one side to the other. Rauber could also in one instance note defects in the formation of one side of the germinal ring. (Fig. 52, Fig. 21).

b. Terata mesodidyma of amphibians

Outside the frog, mesodidymus up to now has been observed one time in Salamandra maculata and has been described by Van Klaussner [33]. Head and

tail are single and position themselves as a knoll perpendicularly over the yolk mass. The portion between them is a saddle-back body split into two halves which encircle the blastopore which is filled with yolk (Klaussner Plate 8, Fig. 49).

Cleavage Formation in the Three Higher Vertebrate Classes

Spina Bifida

Malformations in such early stages as we have been able to observe in amphibians and fishes are not known to us in reptiles, birds and mammals. Their study is more difficult in every respect. First, the fertilization process presents experimental difficulty because it takes place within the female sexual organs. Secondly, it is not possible to secure a large number of eggs which show early stages of malformations because some are encased in an opaque shell (reptiles and birds) and some are internal in the mother (mammals). We, therefore, only see malformation from time to time (by opening a malformed chicken egg or by pathological premature birth) or we learn to recognize them when the malformed creature is at the end of its development and is born. In this case we see only the end result of an earlier disturbance of the development which results in a more or less pathologic and monstrous single organ. The cause, the disturbance itself, its earliest appearance in the developmental process remains unknown to us and can at best be elucidated with a lesser or greater degree of accuracy by comparison with the normal course of development.

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Here the study of malformations of lower vertebrates can be of great value in interpretation. In amphibians and fish we can by patient observation follow a malformation in its development from its beginning stage all the way until all of the organs have been differentiated and are functional. So we see how malformations of single organs in fully developed animals have a genetic origin, how for instance the doubling of the spinal chord and chorda, possibly the intestine and the liver of fishes and amphibians, are caused by the blastopore cleavage remaining open for an abnormally long time. When we now see, in the higher vertebrates, the last sign of a disturbed development such as cleavage of the spinal chord or the vertebral column or of both at once, we are justified in making a comparison with the lower vertebrates. We are no less justified to seek the cause of the disturbance in the identical condition which has shown itself in the beginning of the development process to be a defect in the gastrulation process during the closing of the normal blastopore opening.

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The malformation of three higher vertebral classes and primarily man which are of a "comparative teratology" are known in the literature under the name of spina bifida.

Richter [55, 56] has lately concerned himself with the study of spina bifida in chicks and has experimentally sought to induce the condition by varying temperatures during the incubation period. In one experiment he had three cases of spina bifida in 58 eggs, combined with congenital protrusion

of the brain from the skull, while three other attempts were without result.

In one case the site of the disturbance was in the center between the two forward extremities. In serial sections the spinal cord was greatly enlarged in width and height, the central canal greatly widened and divided into two halves by a dividing sheet which separated itself from the upper wall and attached itself to the lower wall. In more extreme cases the two spinal cord halves in the dorsal region encircle a fossa which is closed over with a fine membrane, "This is especially inclined during further development to hypertrophy downwards colossally." Unfortunately, I cannot find in the foregoing communication of Von Richter any reference to the suppression of the vertebral column, particularly the chorda dorsalis, which is extremely important to the understanding of the formation. The widening of the central canal, the formation of a separating wall, the skin-like closing membrane over widely separated halves of the spinal cord, remind one of similar findings in malformations of frog embryos.

Spina bifida of man, one of the most widely observed malformations of our spines, contributes a rich amount of material to the question under study. Of those who concern themselves with the disturbance with which we are concerned I would mention in particular the investigations of W. Koch [34] and the encompassing, basic treatise of Von Recklinghausen [54].

The numerous, well-known defects of spina bifida of man belong almost entirely to the post-embryonal period, so that disturbances encountered during the developmental period have already arrived at their definitive conclusion. We are naturally interested in the cases of disorder of the greatest magnitude in which the process of formation was least able to approach the norm. In this vein, there is described, fairly often, in the literature bifurcation of the spine which either is restricted to the neck, dorsal or lower portion or on the other hand involves the entire length from the brain to the terminal end (Spina bifida). In some malformations both halves of the spinal cord exist as parallel plates bound together by nerve fibers from which on each side, dorsal and ventral roots arise; in other cases each spinal cord half is enlarged into a tube with a central canal as is also practically always the case in frogs and fishes.

In reference to two exactly described cases Von Recklinghausen remarks: "one must assume that here the embryonal development of the medullary plate followed the unusual course, that each half closed itself off into a tube instead of merging with its partner into one common tube." In reference to his opinion, with which I agree in every respect, this is not a true doubling of the spinal cord substance but only a division. The sum of the mass of the two halves is only equal to the mass of an undivided cord of the same length. Von Recklinghausen refers to the theory advanced by Von Rauber (and before him, Lereboullet) that the split formation is a malformation caused by arrested development, which gets to that stage through a delay in the joining of the right and left halves of the germinal ring and adds that this theory is the most natural explanation of the finding of division of the spinal cord.

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In the cleavage of the spinal cord neighboring organs are also divided. In some cases the soft brain cover pushes itself between the symmetrical spinal cord halves which are transformed into a tube, and in one observation of Von Recklinghausen, a connecting strand between the forward and rear dura mater even pushed itself through the cleavage between the two halves.

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Very often a cleavage of the vertebral column is combined with cleavage of the spinal cord. The spinal canal is open to the rear either in one specific location or for its entire length due to faulty development of the arched halves which by a fault of the spinal process did not fuse together (Rhachischisis posterior). This is the most common finding. Not altogether infrequently, however, another cleavage of the vertebral body presents itself (a Rhachischisis anterior). This presents itself either as a crack through the length of the vertebra, as both halves of the vertebra, which can be more or less rudimentary, may be as far apart as the width of a finger. The defect is filled out with connective tissue. In these severe occurrences of vertebral cleavage the number of vertebrae at hand almost never reaches the normal amount. Thereby the vertebral halves which have been backed up out of their normal location have undergone a greatly disproportionate development. For the most part, they are smaller than normal, some are noticeable only as small seeds of bone, where one does encounter larger pieces they are due to previous fusions as can be noted by the demarcation lines present on some of them (Rindfleisch [62]). Unfortunately, nothing is known about the retardation of the intervertebral discs with their gelatinous nucleus. I would like to urge future investigators to look back upon the important point that doubling of the gelatinous nucleus in forward vertebral cleavages would prove doubling of the chorda.

The most severe case of cleavage of the vertebral column is perhaps the malformation described by Von Braune [8] of the Hallen Museum, which generally presents itself as a double monster. It consists of a hemi-cephalic head, two vertebral columns, two arms, one sternum, and one pelvis with three attached hind extremities. Panum [43] has already conjectured that it is probable in this case that this is not really a double malformation but rather a cleavage which can be classified as a Terata mesodidyma of Oellacher. Therefore, the third extraneous extremity must be the result of a budding induced by the extreme rearward extent of the cleavage of the embryonal layer. I will close the section on Spina bifida of man with a paragraph in which Von Recklinghausen makes the statement from his observations that the underlying reason for spina bifida must be sought in the earliest stages of the developmental process. "The notable frequency of doubling in Rhachischisis and rupture of the spinal cord provides an important basic support for the theory that this sort of spinal cleavage is such an imperfection of skeletal development as occurs when the union of the two halves of the spinal and medullar layers into a simple tube is hindered. When we find the latter regularly divided by a fissure and when we even find a spinal fissure of the slightest magnitude such as that in which there is a hernia of the spinal chord, the myelomeningocele, we must search for the locale of this persistence of the medullary groove in the earliest stages of embryonic development, primarily in a retardation of growth of the

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blastoderm. The muscle and skin plates and also the origin of the fascia seem to be split from the beginning and not to be secondarily ruptured or perforated; also their germinal material lacks in growth potential."

I agree with this observation of Von Recklinghausen and would like to add that that which Von Recklinghausen calls retardation of the growth of the blastoderm is a restraint of the closing of the blastopore in the manner that has been firmly established by observation in amphibians and fishes.¹

So it is that understanding of a great number of malformations is based on basic principles of vertebrate animal morphology. Since these basic principles have up to now not been critically discussed to any extent I would like to explore them in the second chapter in light of recently discovered basic facts.

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2. The Importance of the Discoveries Made From Malformations for Certain Basic Questions of Vertebrate Animal Morphology

a. The blastopore theory

I have designated those peculiarly malformed frog embryos as arrest malformations. Because of this designation it is necessary to undertake in amphibians a more extensive study of the blastopore than we heretofore could make by observation of its normal developmental process. Let us state at the outset that, that which we up to now have designated the blastopore in amphibians and other vertebrate animals in a single stage of development is always only partly the same and subsequently does not retain one and the same form. Then the blastopore changes constantly in the course of development in form, position and extent. Beginning at first in the head region of the embryonic structure, it can be found later in the region of the neck, still later in the chest and the loin region and finally at the tail bud. Therefore, the migration of the blastopore from the head region to the locale in which we encounter it keeps pace exactly with the formation of the primitive vertebral plates.

It, therefore, becomes evident that the blastopore from nearly its beginning closes its forward end by having its edges grow together while it enlarges to the rear and stays open for some time. Any single developmental stage of the vertebrate embryo, therefore, shows us only one stage of the blastopore opening. If we wish to draw any conclusions from the total development of the blastopore we must think of it in all its stages from the beginning of the fusion of the edges onward.

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An excellent example is given us in frog malformations where the delay in closing the blastopore is maximum. Here the blastopore stretches from

¹ Roux also remarks in his preamble to his paper [61], "In the same manner observation on the frog of diastasis medularis, a cleavage of the spinal column spoken of by Von Recklinghausen, supports our findings."

the forward end of the site of the nervous system and chorda dorsalis all the way to the rear and also through the entire hind end of the embryo.¹

The normal gastrulation process of amphibians seems at first glance to contribute little to this, least of all to the timing of the aforementioned phenomenon. We must look deeper to see if worthwhile extension of our knowledge can be gained.

It is well-known that Oscar Schultze [63] and Roux [60] have carried on a lively debate over the gastrulation process of the amphibians over the last few years. Numerous, well thought-out experiments have been brought out in support of their opposite views by both sides. The divergence between the two is so great that Roux attributes the origin of the head to a point on the surface of the egg to which Schultze attributes the tail of the embryo. Schultze designates the dorsal lip of the gastrula in its earliest formative stage to be at a point barely below the mid-line of the egg's surface, which point gives rise to the tail site of the embryo. According to him the gastrulation process takes place in such a manner that the lower or vegetative half of the blastula is taken up by an invagination process directed toward this firm point and by extension of the surface of the animal half into the anterior of the egg and is overgrown by the epiblast. In this manner the middle tube is located in the upper and rightly so named "animal" hemisphere of the blastula.

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Roux in sharpest opposition to Schultze, says the nerve tube is formed on the primitive lower half of the egg. He notes, "We can notice that the material for the formation of the medullary plate on each side through outgrowth from the equatorial edge is pushed down to the underside of the egg and that these plates come together and fuse at their median plane. This fusion takes place in a head to tail direction. In almost the same manner the wandering of the blastopore takes place over nearly 170° of the lower surface of the egg. The gastrulation of the egg seems to take place by an overgrowth of the white under half from both sides of the equator and through bilateral epibole. An invagination takes place only insofar as the nutrient yolk material wanders or is pulled against the roof of the cleavage cavity and rests against it until the disappearance of the cleavage cavity.

Roux proves this theory through cleverly carried out experiments. When fertilized eggs such as were first used by Pfluger [44] are held under tension with the white pole held downward, so that the normal turnings which take place during development are restricted, then the medullary groove, as

¹As has been pointed out earlier, we encounter a similar cleaved blastopore which is also encircled by a nerve ring in the anthozoan members of the animal kingdom. It is also found in its earlier stages of development among the invertebrates in peripatus, annelids and arthropods. In peripatus, the blastopore occupies the entire length of the back and is open for such a time that along its edges on both sides of the cleavage the beginning of primitive vertebral plates are forming.

Pfluger has observed, is formed on the lower surface of the egg and is so oriented that the transverse brain-groove is somewhat in the area of the first appearance of the blastopore while the hind end of the medullary groove is at the last remainder of the blastopore. To prove his point further Roux conducted some "puncture" experiments in which he punctured a particular site on the egg surface with a warmed fine needle and with the passing of time examined the embryo to see where he could find the resulting scar. When the middle of the black hemisphere of the blastula was damaged the scar later was not located in the middle of the black hemisphere of the medullary tube as O. Schultze would have supposed, but was on the ventral side of the embryo. If the first stage of the dorsal lip of the blastopore was disturbed the result was a defect in the transverse brown groove. Injuring a beginning gastrula on the side by the mid-line causes a defect near the middle of the medullary groove when on the other hand an egg at the beginning of gastrulation is injured at a point in the mid-line where the blastopore lies the result is a defect in the caudal end of the embryo. /427

Roux found a third factor in malformations which he described as Asyntaxis medullaris and which I have carefully examined in all of its stages. In these it is immediately apparent that the black hemisphere of the blastula becomes the ventral side of the malformation and that the vegetative half, bound around with the medullary grooves which are not completely pushed over the equator of the egg, gives rise to the dorsal surface.

In the controversy between Roux and Oscar Schultze over the site of blastopore and nerve tube on the surface of the blastula I must take the side of Roux: also I agree with his views on the formation and rearrangement of the blastopore as the reader can infer from the various discussions I have put forth in various places on the blastopore question. On the basis of the experiments and findings of Roux alone it is possible to relate the findings in frog malformations with the normal gastrulation process. At the same time I find in the presentation of Roux an important point which is not properly explored, this is the invagination which occurs during the changing developmental process. Thus the considerably cephalenteric cavity is built from a fine invagination which goes out from the dorsal blastopore margin and is later covered with a simple sheet of cylindrical entoblastic cells. In the same manner in the most severe of our malformations which Roux, mindful that they completely lacked a primary intestinal cavity, also named anentoblastic and described as lacking entoderm, there is sometimes present an elongated sinusoid cephaloenteric cavity which originates by an invagination (compare Plate 17, Fig. 9 and 16 kd). In eggs which develop normally, however, this cavity is considerably larger. Further, we find as the blastopore progresses toward the rear and the vegetative half of the blastula is thereby overgrown, further invagination occurs. One time the yolk material lies against the adjacent inner wall of the blastula causing almost complete obliteration of the cleavage cavity, secondly cell masses grow off the edges of the blastopore between the yolk and the entoderm and become the mesoderm, or in other words, the body cavities push inwards from the edges of the blastopore. So it is shown, on careful analysis, that /428

the simple appearing gastrulation process of amphibians is really in many ways an extraordinary developmental process.

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From our newly gained vantage point we can examine in a clear light three points of amphibian development which I have already mentioned in my monograph on the mesoderm [24].

In eggs of tritons in which the blastopore has already closed to a small hole or transferred into a long groove, I describe, before the medullary grooves appear, on the dorsal surface a groove which runs forward from the blastopore which I have given the name backgroove (Plate 16, Fig. 4-6). In this region the back wall is made up of only two layers of cylindrical cells, the ectoderm and the chorda origin which lies under it (the chordentoblast) both layers are closely bound together as is later the case between the outer and middle embryonic layers (Plate 18, Fig. 1-2). In anurids I describe such a back groove in the region of the cover of the primary intestinal cavity where the chorda originates, which is not always clearly defined. In the back groove, I call attention to the junction line which from the beginning of the gastrulation process has been laid down as the edges of the blastopore progress rearward at a steady pace and fuse together at the median line.

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The second point concerns the establishment of the mesoderm. I believe this to be the result not only of the infolding of the blastopore but also of an outgrowth of both sides of the origin of the chorda whereby cell masses, some of which represent the caelom pockets, push in between the two primary embryonic layers.

Rabl [45] differentiates a peristomal and a gastral mesoblast depending on whether the first or second mode of formation takes place. This differentiation only has significance in the geographic differentiation of the mesoblast according to its relations with neighboring organs at various stages of development. A deeper genetic meaning cannot be ascribed to it. As the chordal origins build themselves in the fusion site of the blastopore edges so the mesoderm is formed through an infolding of the blastopore edges before they have united themselves at the junction line. Everything which in the advanced stage is referred to by Rabl in a topographical sense, as mesoblast was in earlier stages peristomal and became gastral during the fusion process of the edges of the blastopore. In one word: the vertebrates establish their mesoderm chiefly through infolding of the edges of the blastopore.

The third point concerns the growth and formation of the hind end of amphibian embryos. I have already discussed this extensively in my previous publication [24].

There I have designated the hind part of somewhat older embryos and the region of the blastopore as zones of growth and new formation, and have shown through following serial sections how from here through the affixing

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of new parts the mesoderm, the chorda and the medullary tube develop: it says on page 87, i.e., of frog eggs, "When one follows further toward the head region from here (section through the blastopore) one can see the closing of the divided blastopore and by union of both edges the establishment of a median undivided cell mass between the inner and outer embryonic layers. Then one sees the latter become split into an outer and an inner layer. The outer is thickened into the medullary plate and with the dorsal groove becomes ectoblast, the inner layer however, is the chordentoblast. In the same manner there occur between the two, noticeable ectoblast defects along the border of the intestinal portions which are separated from each other the same width as before." The same occurrence comes to light once again in a somewhat older embryo in a similar form on reference to four transverse serial sections (Plate 23, Fig. 1-4) (p. 89). Also for tritons it was shown that the lateral blastopore edges fuse together (pp. 43-44) and the manner in which the ensuing cell mass differentiates into chorda and medullary plate was shown (p. 45).

Through logical inference from these findings and by looking back on the results of Teleost development which were presented by His [29] it could even then be inferred that the chordal origin even in the foremost part of the embryo was laid down by a fusion process of the edges of the blastopore. Even the pure morphological facts indicate this, if one examines the salient points correctly, as I believe I have shown and which later was shown by Pflüger and above all by Roux by experimental studies of the gastrulation process of frog eggs.

At this time I would like to make some further statements about the gastrulation of amphibians.

In the transitional zone of animals in the vegetative half of the embryonal surface or in the marginal zone at the site from which the head end originates a small invagination becomes the cephaloenteric cavity. The invagination enlarges further to the rear, following the marginal zone, whereby the cell masses grow between the yolk and the ectoderm to form the lateral body cavities. At the same time the edges of the blastopore or folding edges grow from their original site at the equator on over the vegetative half of the embryonal surface. They approach the middle from both sides at their near end while the further ends are wider apart so that they describe the form of a horseshoe. As they meet at the mid-line in the course of their growth they fuse together from front to rear. Through this at the junction with the cephaloenteric cavity, first the primary rudiment of the intestine is enlarged, secondly the open end of the blastopore migrates caudally, thirdly the dorsal wall of the embryo develops and it still shows the dorsal groove corresponding to the point of closure.

From the ectoderm in the region of the dorsal groove the medullary plate is formed and the lateral brain groove becomes located slightly forward of this point. From the latter the gastrular invagination has its starting point. From the underlying layer resulting from the fused blastopore edges in the dorsal groove the origin of the chords is formed. That

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portion of the blastopore edge which formed the mesoderm (peristomal mesoblast) by infolding has now become gastral mesoblast in the region of the fusion.

The gastrulation process stretches out for a longer time and progresses from front to back. Constantly the lateral blastopore edges enlarge through which the coelom pockets are developed further in the border zone: they finally close into a ring opposite the site of one of the first invaginations of the primitive blastula. (Origin of the ventral blastopore edges) The vegetative hemisphere has then grown out completely to the side of the open ended yolk plug. The blastopore junction has had a meaningful progression. The still open portion of the blastopore which is now in the form of a ring has migrated from the transverse brain groove all the way to the tail end of the embryo.

The round hole alters by moving its sides closer together into an elongated cleavage in which the fusion as before takes place from front to back, while the open portion for some time longer increases through growth (insertion by division of new cells) and finally participates in the formation of the tail and differentiation of the hind parts, a point to which I shall return later on.

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In this manner the entire dorsal surface of the amphibian embryo with its axial organs from the beginning of the nerve tube and the chorda all the way to its hind end is formed by fusion of the blastopore edges and differentiation processes. Nerve tube and chorda subsequently deposit considerable material across the widening of the blastopore groove.

In our view it can not be difficult to relate the disturbed gastrulation process in previously described malformations to the normal process. Between the two, the main difference is, to be sure, that in the malformations the invagination of the cephaloenteric cavity and the invagination of the coelom pockets in the border zone took place in the way shown in the diagram (Plate 20, Fig. 17), so that the growing together and fusion of the edges of the blastopore in the middle of the back did not take place and therefore the situation diagrammed in Fig. 19, 20, did not come to pass. In the same way it is possible to understand how malformations which at their beginning are so far removed from the norm can in their later development revert toward the normal form and cancel out the disturbances to a large degree. The right and left halves of the back have only to grow together subsequently.

The skillful reader will have noted that my presentation relates to observances which other investigators have made; namely those which His made on teleost eggs and shark embryos some time ago and which he recently has proposed as the concrescence theory. I will take this opportunity to go into the concrescence theory in depth and to give my position on it afterwards.

In his "Letters on Our Body Form", His [29] has said "In the bony

fishes the material for the origin of the back is in the marginal elevation and that those portions of the embryo which lie behind attach themselves along its length and elongate to the rear. The origin of the body is a flat ring (the germinal mound) which divides into two lateral halves which are differentiated into two symmetrical body halves." Lereboullet before His has assigned a similar role to his "embryogenic swelling" and has cited these thoughts subsequently to explain the malformations of pike, as I have previously mentioned (p. 42).

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His has come back to the same theme in other works, as in his presentation on the development of shark embryos [28]. In these earlier works His confined himself to the presentation of developmental laws for the particular object under examination and did not attempt to establish a general theory out of the foregoing results. As a matter of fact he declares the opposite: that he cannot place them in the overall scheme of fish and vertebrate animal development. Not once do the formation of the chorda and medullary tube lend themselves to a meaningful formula. Conversely, His in the last anatomical congress has made such an attempt and in his presentation [31] on the question of elongation of vertebrate animal embryos has put forth a conrescence theory.

From his observation that in the bony fishes and Sea urchins the body is formed through elongation of two lateral halves in that a new portion is added from behind to the forward embryonic portion arising first, he poses the question whether other vertebrate animals in any stage of their development undergo a meaningful elongation of the axial structure. He believes he has found such in the region of the primitive groove and neuroenteric canals. He therefore ascribes to the primitive groove an even further extension to the front, in that the head process arises from it and he designates it according to its importance as a neurochordal groove. "The chorda as the floor of the medullary plate grows out of the two lateral halves which came together in the mid-line. The building and closing of the primitive groove, therefore, goes the entire length of the body with the formation of the chorda and the axial splitting off of the medullary plate to the front."

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Remarkably His denies that the blastopore plays any part in the differentiation of the primitive groove and the neuroenteric canals. That the neuroenteric canal in amniotic vertebrates breaks through secondarily sets it apart from the blastopore previously noted by Rusconi in lower vertebrates or the blastopore of Haekels. "The blastopore", His remarks, "is the gradually decreasing opening which remains free when the more rapidly multiplying upper embryonal layer overgrows the less rapidly multiplying lower half (out of the yolk). The blastopore can be at the same time the neuroenteric opening. This depends above all on where the origin of the embryo is located in the germinal edge in fishes and amphibians. In all amniotic vertebrates the blastopore and the neuroenteric opening are strictly differentiated from each other. The first is the result of an overgrowth deficiency while the latter on the other hand, is a break through, therefore,

the neuroenteric opening of amniotic animals belongs in the same category as openings such as the mouth and anus which also share its axial location." At the conclusion of his treatise His sets the concrescence theory apart from the blastopore question, even though in my view these gave the first understanding of it, and since he puts an embryo-forming fold in place of the blastopore margins his theory is summed up in the following sentences:

In all cranial vertebrate animals the head end of the body is laid down as a horseshoe formed fold of the ectoblast. Between the shanks of the horseshoe lays the primitive groove whose importance to the chorda and medullary plate formation was discussed above. The embryo forming fold may come off from the edge and may pull the germinal margin partly or completely into its locale or it can form separately from the germinal margin. The first is the case in fishes and amphibians and the second in the amniotic vertebrates. In one or the other case various strong forces work in diagonally mediocaudal direction from the primary fold origin, the embryo becomes narrower and also, by gathering in more sideward portions, larger. In lower and in higher vertebrates the axial structure is formed out of two side halves, this making the elongation growth along the mid-line a common phenomenon for all vertebrate animals. In invertebrates this phenomenon finds a parallel in the growth of the primitive streak of worms and arthropods.

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The concrescence theory has in past years and also at the recent anatomical congress been the subject of various attacks. In earlier years Balfour [1b] and Rabl have spoken out strongly against it. Balfour declares that it presents a paradox. For other reasons he uses as the main argument against it that the nutrient yolk in vertebrate animals is located on the ventral side of the body and becomes surrounded by the blastoderm so that in all vertebrate animals with a large amount of yolk the ventral body wall obviously is formed by a closing of the edges of the blastopore at the ventral side. "If His and Rauber are correct," Balfour continued, "then the dorsal walls must also be formed by a growing together of the edges of the blastopore which is obviously a reductio ad absurdum of the entire theory." Balfour comes to the conclusion: "If one studies the theory in the light of comparative embryology, one will find it untenable."

In no less a manner Rabl [45] speaks out against the theory of His. Even the fundamental observations of the theory in bony fish and shark embryos are disputed by Von Kastschenko [32] on the basis of results he obtained when he damaged live shark embryos by operation on the marginal elevation. Kastschenko is convinced, "that the material for the formation of the axial portion of the embryonal body from the beginning is not in the marginal elevation but at the hind end of the germinal disc; that is there, where in fact, the formation of the embryo takes place." In this final conclusion, however, Ruckert [62b] does not agree with him. He has cut off by operation on *Pristiurus* embryos one side of the marginal elevation and has let the embryos develop to an older stage than those of Kastschenko; and then he has observed a lesser development amounting actually to a defect

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in the operated side (by superficial examination).

In contrast to Balfour, Rabl and Kastschenko, various investigators have agreed with the concrescence theory of His, namely Rauber, Roux and Sedgwick-Minot.

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Rauber [46-53] declares the double monstrosity of bony fishes to be a phenomenon produced by the way the germinal mounds come together. It is particularly to his credit that he has attempted to relate this entire phenomenon to the closing of the blastopore.

Roux [60] finds the observations of His regarding the development of a bony fish embryo to be in agreement with the impressions he has found during his examinations on frog eggs. I regard his oft mentioned experiments to be very important and clarifying to the blastopore and concrescence theories.

Lately Charles Sedgwick-Minot [41] has finally declared himself to be on the side of His in an article which is very worthy of examination: "The concrescence theory of the vertebrate embryo." Minot seeks to establish for all vertebrates the formation of the axial portion by fusion in which he duplicates His on the growth junction in the primitive streak. "The vertebrate primitive streak is formed by growing together in the axial line of the future embryo of the two halves of the ectental line." Thereby Minot develops a conception of the entire foregoing process which is reminiscent of that of Rauber, according to him the fusing edges are the edges of the blastopore. The gastrula of vertebrate animals has a blastopore which is elongated greatly and which closes itself toward the rear as the development progresses. The rearmost portion which at times remains open, itself is the blastoporus. "Concrescence is, then, a modified method of uniting the lips of a greatly elongated gastrula mouth."

Looking back on the story of the concrescence theory we see something which occurs in Biology frequently, in that greatly prominent investigators are divided into two segments over its worth. Some declare it to be a paradox from its beginning, while others hail it as a very worthwhile lesson.

I, myself, have not taken the occasion to speak out on the matter up until now. I have, however, made observations in agreement with it such as that in various states of the development of amphibians the forward part of the blastopore is seen to be fused and that from this site of fusion the chorda and medullary plate and mesoderm are differentiated to the front and also that the vertebrate animal body elongates by the addition of parts which are newly built at its rear end and that the blastopore and primitive groove extend themselves from the head end in the same manner.

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I have also included in my textbook the observations of Duval [13] as to how the crescent ridge in the primitive groove is developed and how it grows from front to rear. The full meaning of these truths in relation to the various beliefs is something I have not discussed so far. If I am now to speak out for the first time on the concrescence theory I must first

state that contrary to many other investigators, I find the observation of His that the germinal bundles in fish embryos lay themselves down from front to back to form the axial organs to be a very significant discovery. In the same way I echo his meaningful deduction that this process takes place in the primitive groove and that the head process originates out of the foremost portion of the primitive groove. As something which is wrong, I cite the statements concerning the blastopore and whatever depends on them; and when His attempts to separate the blastopore theory from the concrescence theory I can only say that the latter only becomes understandable when considered together with the former. It is exactly because His failed to clarify the relation of the blastopore to his theory "the development of the vertebrate body by fusion" that one can find the reason why investigators such as Balfour and Rabi have opposed themselves so strongly to his proposition. For the most part I hold the views of Rauber and S. Minot.

After this historical excursion I would like to go more precisely into how the blastopore theory and the concrescence theory are inter-related and how the latter only becomes understandable when it rests on the foundation of the former. The difficulties which stand in the way of the subject will hereby be removed and the blastopore theory will in itself thereby, I hope become clearer. We should study first a purely morphological question which concerns the entire basis of vertebrate bodies from the simplest to the most complicated form; how the gastrulation process of amphioxus can contribute to our knowledge of the fusion of the blastopore along a line which is later noticeable through the site of the chorda and the nerve tube.

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The answer to this is given in the amphioxus monograph of Hatschek [20]. This excellent presentation of observations guarantees the correctness of its statements.

The gastrula of amphioxus can first be compared to the form of a flat, oval disk or a cap in which the subsequent hind end becomes well defined, the ectodermal cells are larger and among them two stand out by virtue of their greater size. Later the wide orifice connects to a small hole which flows into the hind end of the embryo lying in the dorsal surface. Hatschek has painstakingly studied the question of how the narrowing of the blastopore takes place and has come to the conclusion by comparison of single intermediate stages that it takes place by fusion of its edges which takes place in its forward part while the hind end remains open. "The fusion takes place in a line which later forms the greater portion of the dorsal line. The gastrula mouth belongs completely to the later dorsal portion. The gastrula closing along the mid-line duplicates the same process in annelids."

If these presentations of Hatschek are correct, as I surely believe, then in the grown animal the forward end of the blastopore, if we think of it as open for its entire length, becomes the beginning of the nerve tube, as in amphibians. The chord origin would form beneath the fusion site and the medullary plate above it, and the mesoderm to the side of the chord

origin by turning out, so that in these relations a complete agreement would also be made with the results obtained in the frog.

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Amphioxus and the amphibians which are mentioned here as being confirmatory, give us a secure foundation whereby we can ascribe to them the orderly and timely development of the most important organs to the closing of the blastopore. In both, the blastopore is closed at its forward end during development before the chord begins to emerge from the chordentoblast in recognizable form. We are, therefore, not justified in perceiving in these processes a causal connection in such a way that we can say that the chord is formed directly by fusion of the blastopore margins. The truth is rather that a body region is formed through this process, i.e., the cover of the primitive intestine, from which site the chorda dorsalis later arises from origins which are as yet unknown. Thus, the chorda is not easily designated as the axial fusion line but can only indicate this by its location at the site which was once occupied by the opening of the blastopore.

In the same manner, it cannot be shown because of the occurrence of a double chord in delayed closings of the blastopore that the simple chord is the result of the growing together of a doubled chord. Then the doubling of the chord in this case only indicates that the origin material through the delay of the normal developmental process has in some abnormal way been held apart for that period of time in which differentiation of the original material was to begin; it is only a disturbance in the timing of the developmental process which causes the split and therefore holds no historical significance. It can be classified with a similar defect, the doubling of the heart in vertebrate animals with mesoblastic eggs which I set apart in great detail in my text book on the developmental process [25].

The case is different with the central nervous system. Here a series of causes can assert themselves so that the nervous system has been laid down in primitive form as a nerve ring around the blastopore even before the time of its closure, as the anthozoans teach us. The beginning of the nervous system historically precedes the closing of the blastopore and we can truly say that left and right halves of the nerve ring are united by a junction which forms the forward commissur. Both commissures of the central nervous system are junction lines, the forward one being established during closing of the blastopore and the rear one during the transformation of the medullary plates to form the medullary tube.

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A final point, which can now only be briefly explored is the relationship of the blastopore in amphioxus and the amphibians to the elongation of the body. How does this progress to the time when the blastopore encloses only a small site at the rear end? Nearly all investigators agree that in the hind end of the body there can be found a zone of growth or germinal zone through which the body elongates in a similar manner as in the invertebrates and which in its workings can be compared to the vegetation cane at the end of a plant process. The individual investigators, however, have mostly not spoken out on the manner in which this occurs and usually have

not even brought up the question.

Balfour [1b] envisions in the elongation process an unfolding process while His speaks of the coming together of two parts. Balfour says the full amount of mesoblastic segments is arrived at in the manner of the chaetopods, by the insertion of newly formed segments between the last formed segment and the hind end of the body. He uses this as an argument against His's theory of growth, "it would be most surprising if a smaller middle portion of the body would grow in a completely different manner as the end portion. After the nerve tube has closed and has extended through the neuroenteric canal to the intestinal tube it has obviously become impossible for any further extension in length to be the result of fusion."

I think otherwise on the grounds of my amphibian examinations and the amphioxus work of Hatschek. I have earlier already presented the condition that in later stages of development if one examines serial sections through the hind end of the body one finds the same thing whether the embryo has formed 10, 11, or 12 or more primitive vertebral plates. One always finds, if one follows the serial sections from back to front, first the open blastopore cleavage, then the edges lying closely side by side, then that they have fused together into a cell strand upon which is a cleavage the same as in chord and medullary plate, while laterally from this a new primitive vertebral plate is pinching itself off from the surrounding parts. I can envision here a series of related but separately occurring stages of development. Since at the establishment of the tenth primitive vertebral plate a portion of the blastopore closes off and again at the establishment of the eleventh and twelfth, and so forth while the remaining open portion of the blastopore behind the most recently formed segment is always found to be the same size, we are forced to assume that the rear portion of the blastopore has to enlarge through growth to the same degree that the front end loses through fusion. I therefore do not allow that the elongation takes place, as Balfour does, in front of the portion of the blastopore remaining open but rather in this structure itself. Ahead of the blastopore there occurs only differentiation of cell masses which were established by cell diversion in the rear. In amphioxus the zone of growth is differentiated by the presence of large cells which Hatschek has encountered in larvae of various sizes; in amphibians through small celled germinal tissue.

Even though the distance between the open remaining portion of the blastopore and the forward brain portion gets larger with the formation of each new segment, this is not evidence of a germinal zone in front of the blastopore which inserts a new segment between itself and the last segment. We can gather from this that the blastopore actively extends toward the rear through growth. What we see at any single stage as the blastopore is not one and the same unchanged organ. It is merely one of the various stages of an organ which enlarges itself through growth at the hind end and renews itself to the same degree that it is used up at its front end by fusion and differentiation of organs. Therefore, the thoughts brought forth by Balfour against the fusion theory collapse upon themselves.

The execution of the blastopore theory and of the concrescence theory in many respects encounters greater difficulties in meroblastic eggs than in amphioxus and amphibians. Since in these eggs as a result of yolk abundance and the partial cleavage associated with it, many conditions are essentially changed, we must above all become entirely clear regarding the cardinal question, which arrangements of the meroblastic egg are equivalent to the blastopore of amphioxus and the amphibian. It seems to me that this doubling is necessary, considering the uncertainty which His has brought anew into the question already discussed by me in my textbook. To decide this question one must know exactly on what characteristics and attributes the nature of the blastopore depends in the vertebrate animal. Amphioxus and the amphibians have taught us the following:

(1) The blastopore is the orifice of a hollow space which develops through invagination from the blastoderm and permits the intestine and coelom to arise from it;

(2) At the sealing of the blastopore (blastopore margin or lip) the superficial epithelial layer changes continually into the inner layer lining the total cavity or the outer into the inner or middle germ layer by sudden change. Only in the foremost region of the head, where the invagination process has begun, does the establishment of a middle germinal layer not take place, so that here alone the outer and inner layer touch directly for a longer period of time;

(3) When the body sacks have developed through invagination (here later, there earlier) during the gastrulation process then the outer layer changes into the parietal middle layer on the blastopore margin;

(4) In the neighborhood of the blastopore margin the brain and spinal cord develop in the form of a nerve ring, which possesses a break only at the hindmost end where the anus arises from a portion of the blastopore;

(5) The blastopore margins close from front to rear in a longitudinal suture and enlarge simultaneously by a growth taking place from the rear. The distance of the remainder of the blastopore staying open from the head end gradually becomes larger;

(6) The anus arises from the hindmost part of the blastopore. The tail bud develops directly in front of it. (See p. 68);

(7) When a forward portion of the blastopore has closed and the rear portion is still open, one can distinguish from a topographical view a gastric and a peristomal segment of the middle germ layer;

(8) On the roof of the primary intestine along the closing seam of the blastopore margins, the chorda dorsalis differentiates from the chord rudiment and pushes between left and right halves of the gastral middle germ layer, while the peristomal segment is separated into two halves by the blastopore cleavage;

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(9) The middle germ layers or the coelom sacks are therefore established in pairs to a complete extent, with the exception of an area on the rear or ventral blastopore margin, where the invagination produces an unpaired rudiment and serves as a connection of the paired segments;

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(10) The blastopore margins are divided according to their relation to the middle germ layer, into four regions, into a front segment, on which no middle germ layer develops, into the lateral segment, where it is established as pairs in the form of two pockets, and into the rear, unpaired segment.

As it follows from this compilation, the blastopore shows an entire sum of important characteristics in the case of amphioxus and the amphibians. Only in so far as we can demonstrate the corresponding characteristics in the meroblastic eggs may be define the characterized spot as blastopore. I must, therefore, declare myself as decided against a still widespread view, according to which the entire margin of the germinal disk in meroblastic eggs is designated as blastopore margin, nothing is more absurd than such a comparison. The margin of the germinal disk becomes the blastopore only in so far as an invagination arises on it and a blastopore lip is formed on which the outer epithelial layer changes into the inner layer.

That is the case at the beginning of gastrulation only in a very small area, in front of which the oblique brain swelling later comes to lie. By far the larger part of the germinal disk margin shows as before characteristics which belong to the entire margin before the beginning of gastrulation. The cells multiply, whereby merocytes bring about a transition between the uncleaved yolk and the cell material, and in this way a segment of the yolk sphere becoming ever larger receives a cellular coating.

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In order to express the principle difference in the importance of the two marginal areas, I have introduced for you in the second edition of my textbook [25] for the very first time the names blastopore margin and overgrowth margin. Soon afterward, Rabl [45] also agreed with my viewpoint. When we compare a meroblastic egg at the beginning of gastrulation with a frog egg found at the same stage, then the overgrowth margin would correspond somewhat to the marginal zone of the latter, a zone in which the animal cells change into the vegetative cells. If we imagine that the latter are replaced by yolk mass and that this is considerably increased in volume, then we have transformed an amphibian gastrula into a fish gastrula, etc., in the simplest manner.

In the further course of development the blastopore margin and the overgrowth margin show some differences in their relation to one another and to the formation of the embryo, according as we have the egg of a teleost, a sea urchin or a reptile and a bird before us. For a convenient clarification of this I have sketched three series of schematic drawings, diagrams for the gastrulation process of a teleost, a sea urchin and an amniote. In these I have made the blastopore margin distinguishable at its

various conditions of form by a dark black line, the overgrowth margin on the other hand as a dotted line (Plate 20, Fig. 1-4, 7-10).

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What next befalls the teleost development (Fig. 1-4) is depicted in Fig. 1, which represents a somewhat further advanced stage. The blastopore lip, which coincided at the start of the invagination with the margin of the germinal disk and possessed the shape of a crescent, has now received a protrusion directed toward the middle of the disk. This has resulted from the growing together of the left and right halves of the blastopore lips /445 formed first, according to the mechanism first discovered by His. These lips fold up in the direction of a radius which designates the long axis of the future embryo, drawn toward the middle of the blastoderm to the same extent as the entire disk spreads out further in a sheet over the yolk sphere. The part which is formed through folding and coalescence of the blastopore margin progressing from the front to the rear is the rudiment of the head of the embryo which like a knoll, "like a projection" (Raubert) sits atop the germinal disc margin.

Three processes intertwine in the continuation of development for a longer time. First, an ever-larger portion of the yolk sphere is overgrown by the germ layers. The overgrowth takes place at the spot, where the embryo is formed, much more slowly than in the remaining surrounding area of the disk, which immediately becomes clear by comparison of Figures 1-3. While in Fig. 2, for example, the overgrowth margin has moved forward from number 1 to uw^2 , the increase in the area of the developing embryo amounts to only a third or fourth of this.

It can hardly be any surprise since during embryo formation entirely different and complicated cellular displacements, invagination processes, etc. are taking place than is the case in simple overgrowth. That is why we dare not draw any conclusions about a greater measure of cell formation on the more quickly progressing overgrowth margin for the opposite might rather be the case.

Secondly, the embryonic body grows longitudinally by folding up of the blastopore margin, since the neck region, the chest region, etc. are successively attached to the head portion which has arisen first. The presentation of this important process will become more understandable if one distinguishes two different segments, segment ur^1 and ur^2 , from the moment when the head region has been established. I designate by ur^1 the portion of the blastopore which has closed by medial joining of its margins in the blastopore suture; on the other hand I designate by ur^2 the portion of the blastopore lip which turns back at right angles at the hind end of the embryonic rudiment and coincides with the margin of the germinal disk. In short I can call the latter the fixed margin or open portion of the blastopore, the former its coalesced portion. The increase in length of the embryonic rudiment then takes place in such a fashion that the coalesced blastopore section enlarges continually at the expense of the open (or fixed margin) portion of the blastopore, as in amphioxus and the amphibians, since left and right lips move together toward the medial plane and fuse. /446

Through the process progressing gradually from front to rear the open or fixed marginal part of the blastopore lip would soon be used up, if it could not also constantly replace the loss again by growth. The replacement takes place by the fact that the invagination process, which has mainly brought the blastopore lips into existence, continues further on the existing germinal disk margin and that ever new sections of the overgrowth margin are thereby transformed into blastopore margin. The gastrulation process of teleosts therefore extends over a longer interval of development than is usually described and advances on the margin of the germinal disk (at the open part of the blastopore margin) in the original manner which in the forward region of the embryonic rudiment different organs are already differentiating. This reaches its conclusion only by the fact that the lateral blastopore lips are joined with one another at their hind end by the formation of a ventral lip, whereby an invagination margin, closed to form a ring, is produced. At this time the overgrowth of the yolk sphere is also ended and the last remnant of the overgrowth margin is hereby transformed into the closing piece of the blastopore (Fig. 4 a). The tail bud (sk) arises at a slight distance in front of this. The conformity with the blastopore development of amphibians will shine forth itself from my presentation and require no further reference.

In my conception of the gastrulation process of teleosts I agree with His in so far as we both allow for the dorsum of the embryo to form by coalescence of two lateral halves; as for the rest, however, I differ from him in one very essential point. According to my presentation the entire process appears as a modified gastrulation process. The coalescing portions are the blastopore margins and the germinal disk margin participates in the process of coalescence only insofar as it is blastopore lip. On the other hand, according to His the primordium of the body is a flat ring whose width and thickness are at a maximum at one place, the future head end, and at a minimum on the oppositely lying tail end. The material for the head would have to be sought originally at the hind end, the material for the tail at the forward end situated opposite, the "opposite pole" of the original germinal disk, while the material for the tail would be in a lateral region, according to my presentation. According to His, the structures situated in the later medial plane of the body form the periphery of the entire disk originally. The entire marginal fold of the germinal disk is used for embryo formation and this latter is connected with the yolk overgrowth of the embryo in such a way that the thread of embryo material is completed together with the overgrowth. "The two lateral halves of the ring lie one upon another and unite as symmetrical body halves. Only the foremost head end and the hindmost tail end require no coalescence because they result from those sections of the marginal fold which the two lateral halves had closed to form a ring."

Oellacher has already made the not unjustified objection to the theory of Lereboullet, which is very similar to that of His: "If the margins of the germinal disk are supposed to form both body halves, then they must circle the entire egg and then surround a large circle of the sphere at least once; both body halves must once have been quite enormously extended,

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which certainly is not possible." In fact, according to the presentation of His, that the entire germinal margin grows together to form an embryo, the germinal margin would behave like a small rubber ring which one draws over a large sphere. When it arrived at the equator of the sphere, it would be extended to a maximum in order to gradually draw together then on the opposite surface. If the entire germinal margin were blastopore margin, then it would have to stretch enormously for a long time and then narrow again significantly. This difficulty falls away completely in my form. And so I can probably say that the concrescence theory of His appears again in a fairly substantially changed form in the blastopore theory.

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Sea urchins bring about a transition between the gastrulation process of teleosts and amniotes. In sea urchins the formation of the embryo also goes on for a long time through coalescence of the blastopore margins in the manner carried out more precisely for the teleost egg. (Diagrams 1 and 2 can therefore also serve for the first stages of sea urchin development.) Then, however, a modification of the process becomes necessary owing to the considerable size of the yolk. The overgrowth margin of the germinal disk has not been able to envelop the yolk completely up to the time when the lateral blastopore lips already unite toward the rear during the formation of a ventral blastopore lip. As a result changes characteristic for the sea urchin now appear, changes which I have attempted to reproduce schematically by Figures 7 and 8.

Overgrowth margin (Fig. 7 uw⁴) and blastopore margin (ur²) are separated from one another (Fig. 8 a and uw⁵). The embryonic rudiment is detached from the blastoderm margin. Behind the embryo the overgrowth margin forms an enclosed ring (uw⁵), inside which the yolk (d) lies exposed for some time until it is also overgrown by progressive diminution of the ring. Balfour has added the name yolk blastoporus to this ring and discovered a part of the blastopore in it. As I have already said earlier, I consider this view, which is widespread in the literature, to be erroneous. For, in the first place no opening at all is present at the so-called "yolk blastoporus," through which one can get into a hollow space, as is the case at the blastopore, as long as it hasn't coalesced yet. One comes through the overgrowth ring into the yolk mass which one can imagine replaced by a mass of yolk cells in which the overgrowth margin is lost. The comparison with the blastopore of amphibians is no less unsuccessful. For this shows the characteristics of the blastopore enumerated above when it has formed completely. It is therefore not a deficiency of overgrowth or, as His expresses it, the gradually diminishing opening which remains free when the more quickly furrowed upper embryo half overgrows the more slowly furrowed lower embryo half, or the yolk. It is rather an invagination deficiency (blastopore).

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From the sea urchins it requires a short step to understand the situations of reptiles and birds. The blastopore margin of the germinal disk is the primitive streak; this closes here, however, extraordinarily early to form a ring, in that it is transformed into the primitive furrow

as Duval has described the details, and is thereby separated from the overgrowth margin for a long time before medullary folds, chord and primitive segments are established in the foremost region of the blastopore ring. The embryonic rudiment therefore gives up its fixed margin position very much earlier in the amniotes than in the sea urchins and is soon removed equally far from the overgrowth margin into the middle of the germinal disk. Figs. 1, 9 and 10 can serve as diagrams for this abbreviated and accelerated sort of blastopore formation and of yolk overgrowth which has early become independent of it. In the following sentences I briefly summarize once again the difference between blastopore formation (gastrulation process) and yolk overgrowth of teleosts, sea urchins and amniotes.

In teleosts, the overgrowth margin of the germinal disk has almost completely enveloped the yolk, even before the blastopore has secured its distal closure. As a result, the last part of the overgrowth margin is used up in the formation of the blastopore margin, when it still encloses only a small ring at the hind end of the embryonic rudiment. Therefore, the embryo remains margin-fixed to the last, as one would say. In the sea urchin blastopore closure occurs even when the overgrowth margin still has not covered a small field. From this moment the embryonic rudiment, up to now fixed margin, is detached from the blastoderm. The overgrowth ring closes separately from the embryo. Finally in reptiles and birds the separation of blastopore margin and overgrowth margin of the germinal disk takes place extraordinarily early so that the embryonic rudiment soon comes to lie more in the middle of the blastoderm, removed from the overgrowth margin.

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I have gone into the yolk overgrowth so minutely because confusion exists in the literature regarding this question. I quote only the statement of Balfour that in all vertebrates with large yolk the ventral abdominal wall is obviously completed by the closure of the blastopore lips on the ventral side. I draw attention to Balfour's argument through which he seeks to carry His's concrescence/theory ad absurdum, that by acceptance of this theory the entire dorsal as well as the entire ventral abdominal wall of the embryo would have to be formed through coalescence of the blastopore lips. Finally I draw attention to the newest ideas put forward by His, referred to above.

It would be a profitable beginning if, further from the viewpoint developed here, the germinal margin of meroblastic eggs would be first extensively investigated according to its locally and temporally different manner of development, whereby still further light would be spread regarding many points which I have analyzed more theoretically here.

I conclude the section on blastopore theory and concrescence theory while emphasizing some points of comparison between the blastopore region of amphibians and the blastopore region of reptiles and birds (See also Textbook of Developmental History [25]).

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The blastopore of amniotes is the primitive furrow, whose margins fuse early in a medial suture to form the primitive streak to which all three germ layers are connected to one another for some distance. The head process is still to be counted towards the blastopore region and is probably that part on which the fusion of the blastopore lips takes place at the beginning of gastrulation in the manner described for amphioxus and amphibians. Accordingly, the origin of the blastopore region in the developed portion would also have to be sought here at the origin of the chord and in the mid-brain region.

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The importance of the primitive furrow for the formation of the chord, the middle germ layer, etc., is the same as the importance of the blastopore margins in Amphioxus and amphibians. In its first origin the primitive furrow belongs to the head region of the embryo; since it differentiates there into the different axial organs, it grows further at its hind end, the growth zone, by insertion of newly formed embryonic cells. According to the degree of development of the germinal disks it is found in the neck region, the chest region, the lumbar region, i.e., its distance from the beginning of the medullary tube becomes an ever greater one with the age of the embryo. This is not interpreted according to Balfour's hypothesis by saying that newly formed body sections shove in by intussusception in front of the primitive furrow but by the fact that the cell material of the primitive furrow itself, including its environment, is transformed toward the front into medullary plate, chord, primitive segments, roof of the intestine, while it enlarges toward the rear by growth.

At the hind end of the primitive furrow there is frequently a small opening, the much discussed canalis neurentericus, through which one reaches from the ectoderm side into the intestine. His says of it, "it is established with great sharpness that it first breaks through secondarily in amniotic vertebrates, it is therefore a break opening and belongs in the same category of openings as the mouth. Therefore, it cannot be compared with the blastopore of lower vertebrates known since Rusconi or with the blastopore in the sense of Haeckel."

As before in this question I persist in the viewpoint taken earlier. As one has to distinguish in the blastopore region of lower vertebrates a closed and an open portion, which latter occupies another place in individual stages, so must one also distinguish in amniotes. Here is the open part of the canalis neurentericus. If an opening at the end of the primitive streak cannot be demonstrated at all stages of development of the germinal disk, this is explained in an easy manner from the circumstance that the blastopore margins lie close enough to one another to touch. If they then withdraw from one another again at other stages, then an opening appears. In this case, however, a break or the formation of a new opening, as represented by the permanent mouth, has not taken place.

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At a later stage the anal groove can be distinguished in amniotes and the hindmost end of the primitive streak, somewhat behind the place where

the neurenteric canal lies. The tail rudiment of the embryo develops directly in front of it. Like the anal groove of malformed frog embryos, the anal groove signifies to us the hindmost end of the blastopore.

The next chapter will deal with that in detail.

B) Tail and Anus Formation of Vertebrates

Arrest malformations of frog embryos are also very instructive for tail and anus formation. In the highest degree of retardation, in which the entire blastopore region is wide open (Table 16, Fig. 1) the anal region can already be recognized as such, in that there forms at the hind end of the embryo in the ectoderm a furrow (ar) which is bordered by small folds and leads into the pelvic intestinal cavity. As a result the blastopore margin shows here a break, an indentation. On both sides of it the blastopore margins are significantly thickened by the fact that cells are engaged in lively growth and produce a small celled germinal tissue serving longitudinal growth. The cells form two swellings (sk) on both sides of the anal groove and these folds were designated as tail buds with respect to their further development. The nerve ring, which is otherwise established in the entire periphery of the blastopore as the medullary plate, is interrupted at one small spot by the anal groove. Therefore on the blastopore margin we have to distinguish a larger neural segment and a smaller non-neural segment, which is very important for further development.

In other malformations in which the blastopore region has experienced a partial closure, the important fact can be established that the blastopore closes not only successively from front to rear but that a second place of closure develops on the hind end independently of it, and indeed in the following manner (Plate 16, Fig. 9-11, 13, 14).

Above the anal groove both tail buds lie with their medial surfaces on one another and form, by fusing, a broad transverse elevation which consists of a small celled tissue separated, however, into the different germ layers. The hindmost part of the blastopore, which later becomes the anus (ar), is separated from the remaining part by the elevation. The closure of this part has become arrested in an anomalous manner in the malformations. In a series of frontal sections (Plate 17, Fig. 13, 14; Plate 18, Fig. 8-11, 25) the suture formation could still be clearly recognized on the hind and later ventral sides of the elevation; first a furrow and a seam (n) are present and in their region inner and outer layers are connected for a distance (Plate 17, Fig. 13 n, Plate 18, Fig. 2 and 10 n); secondly, at the extreme end a black pigment line passes through the small celled tissue, produced by the coalescence of ectodermal surfaces (Plate 18, Fig. 25 n).

If fusion is complete, as it is in the normal course of development, then an unpaired tail bud arises early from the paired buds and it lies upon the anal groove from above. However, in many malformations fusion is

only partial and concerns only the region above the anal groove, while beyond it the tail buds remain separated up to a more or less large terminal piece (Plate 16, Fig. 11, 13, 18, 19 s).

On the tail bud we can distinguish a ventral and a dorsal surface and accordingly a ventral and a dorsal tail suture as well. The former proceeds from the tip of the tail up to the anus, the latter from the tip of the tail up to the part of the blastopore remaining open as a result of retardation. The former belongs to the non-neural part of the blastopore region, the latter to its neural part.

So far as the anus is concerned, four different stages can be distinguished in its entire development. In the first stage it appears only as the hindmost segment of the entire blastopore (Plate 16, Fig. 1 ar).

In the second stage it has separated from it as a special opening in this way, that the tail buds have formed at the hind end of the neural segment of the blastopore margins and have joined to form an unpaired tail rudiment (Plate 16, Fig. 11, etc. ar). A penetrable opening is, for the most part, not to be found on cross-sections through the anal region, because the walls touch directly. However, in the second stage the original derivation of the blastopore can still be recognized in the relationship of the germ layers (Plate 17, Fig. 5; Plate 18, Fig. 11 ar). As in the blastopore all three germ layers are in connection with one another at the anal position. At the anal lip the outer layer changes into the parietal middle layer and inwards from there the visceral middle layer is transformed again at the intestinal lip into the intestinal gland layer. Thus there still exists at this stage, precisely taken, no direct connection of the outer germ layer with the inner one, but only through intervention of the middle layer. Even if an opening is present, we come through the anal groove first into a disappearing small remnant of the primitive intestine. For we must designate as such the space situated in the region of the middle layer, a space through which one can penetrate not only into the coelomic sac but also in the secondary intestine.

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This situation changes in the third stage of development. In the anal region especially the middle germ layer is separated from the connection described above, first in front on the anal lip from the outer germ layer and then on the intestinal lip from the intestinal gland layer. The coelomic sacs have constricted and closed. As a result outer and inner germ layer now come into direct connection at the point of detachment and border the anal tube in those cases where an opening has lasted. The anal tube now leads directly and immediately from the outside into the hindgut since it pierces the middle layer. For the most part, however, an opening is still lacking because of the condition emphasized above. Outer and inner germ layer adjoin directly as a result of the detachment of the middle layer, but still form together an epithelial closure, the anal membrane or anal cord. We speak of an anal membrane (Plate 18, Fig. 9) when the anal groove (ar) is separated from the cavity of the hind gut (ed) only by a thin lamella, which consists of a simple stratum of entoderm cells and of

ectoderm cells, as is the case in the normal course of development of the frog. On the other hand an anal cord develops in those cases in which, as many malformations, the cavity of the hind gut and the anal groove have moved somewhat further apart and thus a thicker epithelial band appears in place of the thin epithelial membrane, if the middle layer is separated from its connection.

Finally in the fourth stage of development the anus becomes penetrable in that the cells in the middle of the epithelial closure membrane withdraw from one another. At this time or even before in many malformations the furrow leading from the ventral tail root to the anal groove, which frequently is enclosed by high folds (Plate 19, Fig. 1 a, 5 af), has closed to form a short tube (Fig. 2, 22 ar). In this case one then reaches the hind gut through a more or less long, ectodermal anal tube, which is situated at the root of the tail at the beginning of the ventral tail fin. Even in normal development such an ectodermally attached part seems to me to form.

I have briefly summarized the conclusion to which the investigation of tail and anal formation in the malformations of the frog has led me. They agree essentially in a gratifying manner with the results at which several investigators, especially Schanz, Gotte, v. Erlanger, have most recently arrived concerning the rudiment of the anus in normally developing frog embryos.

For a long time great confusion has prevailed in the question of anal development. As is known even here three different views have been advanced.

According to the older conception the anus, like the mouth, is supposed to be a new formation and is supposed to result from the skin sinking to form the groove at the hind body end and later breaking through into the hind gut. According to a second view, which has gained by a study of *Petromyzon* and amphibians, the entire blastopore is supposed to become the anus directly. Finally a third group of investigators (Schanz [62c], Bonnet [3], Götte, Erlanger and others) also assume a relation of the anus to the blastopore, but only to the hindmost part of it. The blastopore can be divided into two openings, into a forward one which is absorbed into the hind end of the nerve tube (canalis neurentericus, chord blastopore) and into a hind opening which becomes the anus (anal blastopore, anal canal).

As far as the amphibians especially are concerned, I can agree in general with the presentation given by Gotte [19] and Erlanger [14].

Gotte permits the blastopore, when it has been transformed into a longitudinal fissure to close in the middle by coalescence of its lips (prostoma suture). Beneath the place of closure a small segment of the blastopore is preserved and it usually loses its lumen, in order to open again later and to form the anus. The remnant of the blastopore situated dorsally from the place of coalescence represents the canalis neurentericus,

which soon is overgrown by the medullary folds and is thereby enclosed to form the nerve tube. The tail, on which the prostome suture is drawn out from the tip up to the anus, arises from the point of coalescence.

In a similar fashion von Erlanger summarizes the result of his investigations in the sentences: "if one compares the results of the works by Schanz and Morgan [40] with those of my work, then one will probably come to the conclusion that the anus arises from the most ventral part of the blastopore, while the most dorsal part forms the neuropore and the canalis neurentericus. In anurids there is the added situation that the place of the blastopore, from which the anus arises, is closed temporarily and the anus is opened only later through a breach, while the most ventral part of the blastopore is never supposed to coalesce in the urodeles. It may thus appear justified to consider the anus formation as secondarily modified in the anurids."

In my work concerning the middle germ layer of amphibians [24], I have illustrated six sections from a series through the region of the blastopore of an older frog embryo which completely, correctly reproduce the processes taking place here. Thus Fig. 5, Plate 8 shows a section through the place of closure through which the canalis neurentericus, situated dorsally from it, and of which a section is illustrated in Fig. 1 is separated by the ventrally situated anal groove (Fig. 6). At the place of closure suture formation appears clearly in that the outer and inner germ layer change directly into the middle layer at one small spot. The anal groove is separated from the hind gut by a thin, epithelial closure membrane, as is the case in the third stage of development B recognized by me. Then, of course I have discovered, as has Gotte also, a new formation in the anus according to the prevailing conception, because the intermediate stages in development had escaped me and its belonging to the blastopore region was overlooked. However, I would now like to refer to the illustrations since they reproduce very well all of the appropriate conditions. Attention is directed also to Plate 7, Fig. 12-14 (l.c.).

Now if we compare the normal and disordered development with one another, then the latter can also contribute here to the understanding of the former things. It spreads more light especially on tail formation and on the morphological orientation of the tail to the rest of the body. I would like to emphasize, as an important result, that the tail can be considered not as a direct extension of the entire body but only as a continuous structure of the dorsal surface towards its origin. The entire surface of the body situated ventrally from the blastopore is not at all concerned with its origin. The tail therefore appears as a continuation structure of the body, which takes its origin from the blastopore region and grows out over the hindmost part of it, the anus. It thereby stands in contrast to the entire remainder of the body. Therefore, we also find in its construction only dorsally situated organs, nerve tube, chord, primitive segments, while abdominal cavity, sex organs, kidneys do not continue into it. Whether we may speak of a real post-anal gut appears questionable to me. Of course,

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the inner germ layer elongates as a cord into the tail rudiment, as a protrusion of the pelvic intestinal wall. For the most part however, formation of a cavity does not appear to occur, and later the cord disappears and dissolves into other tissues. It will probably have functioned as an intestine in no vertebrate so that I suggest that we do away with the name post-anal gut entirely and replace it by the designation "entoderm cord of the tail," which leads to no false conceptions.

Since outer, middle and inner germ layers meet at the blastopore margin and produce the medially situated organs, nerve tube, chord, primitive segments, the rudiments of all these organs are also bestowed upon the tail bud. If the tail bud then elongates and appears as a continuation above the body towards the rear, this takes place in the same manner as the entire body has grown in length. From the growth zone, which has moved to the tip of the tail, primitive segment is attached to primitive segment and can thus yield, at times, an appendage of the body of very considerable length and organized into metameres.

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The tail arises from a paired rudiment since the hindmost segment of the left and right blastopore margin coalesce above the anal groove to form the tail bud. It would be unjustified if one wished to deduce from this, the conception of primitive forms of vertebrates which had possessed double tail processes on their hind ends. Obviously fusion of the blastopore margins is an older process than tail formation. Only after fusion has occurred at the rear of the body end does the tail process develop from the area of the body which has become so simplified. Only under abnormal conditions can the original duplication of rudiments come into play in the developmental process, then especially if by any kind of disturbing cause, the fusion of the blastopore margins to form a simple tail bud is prevented at the right time. Then, as we have seen in several examples, double tail buds form and these grow forth over the end of the body separated from one another and elongate to form two long half-tails composed of nerve tube, chord and primitive segments. According to their origin these are to be arranged in the category of arrest malformations. Even here, a fusion process can still be introduced additionally and it begins from the tail root. The origin of the tail from a left and right rudiment material seems to be able to occur even with mutilation. Perhaps this is associated with the fact that a doubling frequently takes place instead of a simple replacement of the lost end upon cutting off of the tail end of amphibians and reptiles. Lizards with double tails are familiar enough. But even from pelobates larvae Bruch [9] has described cleavage of the tail end during doubling of the chord as a result of mutilation.

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As far as amniotic vertebrates are concerned, the anal groove is established among them at the hindmost end of the primitive furrow (Gasser, Kolliker, Bonnet, Strahl, etc.). The tail bud later forms right in front of it, whereby the anus finally moves beneath the root of the tail more on the ventral side of the body. The developmental processes appear in general

to correspond to those described from amphibians. Therefore we can also extend to the amniotes doctrine that among them, the anus is derived from a small rear section of the blastopore, and that the tail takes its origin from the region of the blastopore area situated in front of the anus, after a fusion of the blastopore lips (margins of the primitive furrow) has taken place.

3. The Coelomic Theory (Reply to Gotte, The Chord Canal)

In his developmental history of the river lamprey, Götte [19] has briefly instituted a violent polemic against my presentation of the formation of the middle germ layer of amphibians, and chiefly against the coelomic theory. As I have already in other cases I would have kept silent here as well, leaving further discussion to the future--for to what extent would our already voluminous literature swell if everyone wished at once to take up the gauntlet, thrown down over the rich number of controversial subjects?-- if I had not been led in my investigations directly to the object in dispute, the development of frogs. Thus I find myself put into a sort of forced position in which I resolve to make some observations, even if unwillingly.

Gotte summarizes the conclusion of his critique in the sentence: "The alleged observations of O. Hertwig concerning the origin of the mesoderm of amphibians are nothing more than an empirical, unfounded--and according to actual observations--erroneous assumption." /460

Since I myself have not investigated the gastrulation process of frog eggs recently, although I am convinced that much can be determined even more clearly by the use of improved aids and newer viewpoints, than have happened up to now, I must in the first place limit my reply by emphasizing that Gotte himself submits no new factual material but believes the matter settled with the cheap expression, "he will have to maintain his earlier data entirely and completely on the basis of renewed investigation on bombinator and pelobates." In the second place, I refer to the work of Schwink, "Concerning the Development of the Middle Germ Layer and of the Chorda Dorsalis of Amphibians." [65] which appeared of course one year before Götte, "River Lampreys," but is nevertheless not given a word of mention by the latter. Schwink has investigated very extensively not only urodeles but also anurids (Rana and Bufo), and on the basis of the comparative studies comes to essentially the same conclusions as I. Every reader will immediately notice the great agreement between the pictures of urodele and anurid development given by Schwink and by me, and he will thus gain the conviction that it is not a question, as Götte says, of "alleged", but "actual" observations.

To no less extent do I call attention to the good illustrations in the investigation of Erlanger [14] and to his article [15] which has just appeared in the Anatomischen Anzeiger, where it says: "This work would still have deserved mentioning principally and especially in the review of

the coelomic diverticulum of the primitive intestine, which I have seen with the greatest clarity in the manner ascribed by O. Hertwig in anurid species investigated jointly by me, and perhaps have not illustrated clearly enough." He certainly throws light upon the accuracy of the renewed investigation by Gotte, that he has not yet gotten to see coelomic diverticuli. Finally, in the third place, I emphasize that I was the first to call attention to the significance of the findings for the development of the middle germ layer (lip formation on both sides of the chord rudiment, the lack of intestinal layer beneath the latter, the mechanism of origin of the peristomal mesoblasts). These findings have been confirmed by numerous investigators for other vertebrate classes (sea urchins, reptiles, mammals). (Balfour, Ruckert, Rabl, van Beneden, Ziegler, etc.).

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Gotte supposes, (in part owing to my "own tacit admission") that I now consider incorrect the facts which make my protrusion theory valid. I can now provide the explanation, briefly and convincingly, for this reason: even today I still maintain the viewpoint developed earlier, that the middle germ layer of vertebrates is formed by an invagination process in the vicinity of the blastopore and that the abdominal cavity is derived from primitive intestinal divercula.

I could allow the matter in dispute to rest, if the peculiar contrast of "alleged" and of "actual observations" were not found in the sentence by Gotte cited above. What Gotte understands under the heading, "alleged observations," is revealed more precisely to us by information in two places when he says, "principles of a corrected, so to speak, observation," or says, "that O. Hertwig adjusts the developmental historical observations according to the end results of the course of development." Gotte surely wishes to attribute to me, the strange purpose-- and I wish to allow him to speak again-- "of wishing to introduce a new perception theory into comparative developmental history, namely, the construction or actual development of a body part according to the end results, when it isn't that way, even in contradiction with empiricism." Afterward, a sentence was printed at once in Sperrschrift: "This implies nothing further than withdrawing the empirical foundations of zoology and natural science and replacing them by a priori conceptions, and with it throwing the inductive methods of modern natural science in a trash pile."

My answer to this turns out very briefly, What I have to say is expressed in three words: thoughtless, gross supposition!

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It is probably not difficult to infer from my investigations that even in my eyes, the facts decide between value and worthlessness of each theory. Have I not in every one of my publications, convinced of the complete value of sentient perception as the indispensable foundation for further thought, drawn a hair-sharp boundary line between objective facts--descriptions of observations--and between the conclusions drawn from the observations? I can err in the observations, err in the conclusions, but I have never communicated, "alleged observations," or drawn conclusions which were not

founded on observations.

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As a conscientious observer does not fear to communicate his observations, always expectant that soon someone will come along who has observed better and more sharply with more talent than he, so should he also, without fear, draw from his observations the conclusions appearing to him, even at the risk of later becoming convinced of a false conclusion. As the history of all science teaches, science not infrequently grows even through errors to which truth is contrasted ever so brightly.

I herewith conclude a polemic to which I have admitted myself unwillingly and against my custom. Since I am therefore still entirely convinced of the correctness of the coelomic theory for vertebrates, I shall touch upon a subject closely associated with it, even at the risk of again exciting the displeasure of my Strassburg critic.

At this time the relation of mammalian development to the coelomic theory is mostly unclear: the structure known under the name of chord canal presents some special difficulties. There seems to me to be a way to show now how the occurrence of a chord canal can be explained in a simple manner from the situations of the remaining vertebrates. The diagram (Plate 20, Fig. 16) should serve for better illustration.

In the vicinity of the blastopore of mammals or in the vicinity of the primitive furrow, which is the same, there are found two lip structures in the closest proximity: 1) the blastopore lips, on which the germ layer changes into the parietal middle layer and 2) the intestinal layer lips, on which intestinal duct layer and visceral middle layer change into one another. With blastopore closure only the blastopore lips now coalesce as a rule in the different vertebrate classes, while the lips of the intestinal layer remain separated from one another by a small distance and thus border the chord rudiment on both sides, which forms on the place of closure and as a result takes part in defining the blastopore. According to my view a small deviation from this type of formation now takes place in mammals in such a way that even the lips of the intestinal layer come to lie together from the beginning of their formation in the medial plane (Fig. 16 B, dl), and if they do not also fuse with one another, as is later the case with the blastopore lips (ul), then they still touch closely and eventually even become glued together. Thus a more or less long, narrow canal (chk) occurs beneath the chord rudiment (Fig. 16, B ch) and it opens behind onto the surface of the primitive furrow through the neurenteric opening (Fig. 16 A), and is united with the blastoderm cavity toward the front (Fig. 16 d) through a second opening.

Of all the investigators who have been occupied with the first stages of mammalian development, van Beneden [2a] has probably studied the chord canal most extensively and precisely in its different relations. As I find, all his data fit my explanation. Thus van Beneden notes--compare this with the diagram sketched by me-- 1) that the coelomic fissures open at first

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into the chord canal, 2) that the chord rudiment proceeds continually laterally in the upper layer of the mesoblast, 3) that the floor of the canal is transformed into the under layer of the mesoblast. Van Beneden says that the chord cavity corresponds to the archenteron or the intestinal canal. According to my view it would represent only a temporarily separated, small part of it while the remaining larger part evolves into the cavity of the blastoderm, in which the yolk has re-formed, as is now generally assumed. The chord canal is just a more incidental, chance structure, to which no special significance belongs. Therefore, it opens even later, as van Beneden describes, at first in its middle into the blastoderm cavity through numerous orifices which soon run together to form a single longitudinal fissure. I would like to express it thusly, that the lips of the intestinal layer situated by one another move apart at a certain stage. Finally, the entire chord canal is united with the principal part of the primitive intestinal cavity (blastoderm cavity, van Beneden) with the exception of the typical canalis neurentericus.

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I believe I may discover therein the basis for the formation of this modification in mammals, that at the beginning of the invagination process all fold structures are crowded together in a very narrow space in Hensen's node and as a result their lips touch right from the beginning and only later change into the normal position. The agreement with the remaining vertebrate classes would be produced herewith.

4. Relations of the Blastopore to Different Forms of Malformations

After it has been established in numerous ways that the monstrous frog larvae described in this treatise, as well as the mesodidymi of bony fishes and the disorders known under the name of spina bifida, are, according to their nature, arrest malformations, which have arisen by delayed closure of the blastopore and the splitting of the axial organs connected with it, I wish to direct attention in this section to some other relations of the blastopore to different forms of malformations. In so doing it will be shown that from my standpoint the first beginning of double and triple formations is even more completely clarified, as has already happened through the excellent treatises of Rauber [46-53].

First of all I must do away with what I believe is an erroneous conception. Roux [61] discussed at the conclusion of his oft-mentioned treatise the question whether, in the case of frog malformations, his hemiembryones laterales, produced by blastopore cleavage, each half might not be able to be restored to form a complete rudiment and whether, in this manner, double malformations might not be able to come into being from a single egg. Roux considered it possible that the semimedulla, the semi-chord and the germ layer situated beneath the chord can be restored by postgeneration in those places where they impinge upon the yolk cells directly. "Every antimere would postgenerate in the yolk during its metamorphosis, progressing spatially successively, a wide piece of the other

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half until both structures met in the medial plane of the entire egg. In this region of contact the pieces of lateral body halves formed additionally must then meet parts corresponding with each other inasmuch as the formation of both sides takes place approximately symmetrically. In a secondary manner we thus obtained incomplete double structures which correspond to the law of doubled symmetry of organ rudiments named by me and clearly described already in the article by Meckel. In this manner especially duplicitas dorsalis, which has not yet been observed during its formation, would be able to arise and, of course, more frequently duplicitas dorsicaudalis, less frequently dorsicephalica."

This hypothesis stands in opposition to the facts. For in the first place we see that in amphibians and fishes the axial organs, which haven't reached coalescence in the medial plane, are transformed at later stages still into a state more or less corresponding to normal; in the second place every part already possesses the counterpart belonging to it, so that a deviation of an unregulated sort from the norm exists only in the spatial arrangement and what is not lacking need not be postgenerated; in the third place the yolk cells are not to be regarded as an undifferentiated egg substance awaiting differentiation but even by this time as part of the stock of an organ rudiment, namely the intestine.

Thereupon Klaussner [33] soon attributed a very wide importance for the clarification of multiple formation to the hypothesis expressed rather incidentally by Roux. He divided the latter into two large groups, 1) into those which arise through primary plurality of the rudiment and 2) into those which arise through fission with postgeneration. He proposes to label the former as twin, triplet and quadruplet formations. The latter as double, triple and quadruple formations.

In contrast to Klaussner and Roux I am now of the opinion that among the vertebrates multiple formations arise not by way of postgeneration of half rudiments which are already relatively widely differentiated, but that the conditions for two or more rudiments are already present in the embryo at early stages. In teleost embryos Rauber [50-53] has advanced the proof for this view in that he followed the origin of double and triple monsters back rather far, namely to the stage when the germinal disk is still rather small and the first rudiment of a head process can be recognized. Rauber remarks, "In the usual case, upon gradual enlargement and extension of this embryo over the vitelline sphere, a single, at first forward, embryonic rudiment reaches formation. Now, instead, in the case of an axial increase, two or three embryonic rudiments appear simultaneously." He designates these in other places also as "advances of the germinal ring", which go out from it in a meridional direction and are vertical at the blastopore. As a consequence of this, "the tail portions of the germinal orifice are always turned to, the heads turned away from it." According to Rauber, "the formation of several rudiments always follows from this as a "pluriradial" and stomatogenic" formation and depends at the same time on a division of the germinal disk region and in the latter instance of the germ substance." Different forms of monsters result according to the position which the

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numerous rudiments occupy on the germinal ring, according as they lie nearer or more distant from one another. Rauber gives a very conclusive and enlightening presentation of all these conditions.

Yet in one point, which is of considerable significance, I cannot agree with him. Rauber regards the entire margin of the germinal disk as the blastopore lip, which I have already attempted to demonstrate above (page 443) as not proved correct. He thereby permits the multiple formations to develop not only from a single cell but also from a single gastrula, a gastrula in which two or three medullary rudiments form, contrary to the normal course. Thus Rauber remarks [52, page 163] in a manner not to be misunderstood about a double formation of Forellen eggs: "While only a gastrula is present, there exists a double neurula," under which name he designates the stage where the rudiment of the medullary tube begins to appear in the embryo. And in another place he says: "A multiple neurula thus develops in the multiple formations from a single gastrula." (46, page 84).

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On the other hand according to my concept multiple formations can be traced back to multiple gastrula invaginations. Since now in the different vertebrate classes, depending on the properties of the egg cells, which are determined first of all by their yolk content, the gastrulation process takes very different forms--I call attention, for example, to the fully discussed relation of the blastopore lip and the overgrowth margin to one another among teleosts, selachiae and amniotes--then it can be expected that the kind of gastrulation process will manifest itself by the special development of multiple formations in the individual vertebrate classes. In this regard Rauber has also directed attention already to fundamental, regular relations between normal and "pluriradial development." Starting from a statement by Hunter "That a characteristic sort of malformation is especially peculiar to every sort of animal", he adds: "In so far as the statement contains truth, the instigating cause can be traced back essentially to differences in normal development. The different spatial relations between egg and embryonic rudiments, the difference in the degree of use of the germinal ring for the embryonic rudiments, the presence of total or partial cleavage, these are the conditions which cause the essential differences of multiple formation of the different vertebrate divisions, without the nature of multiple formations undergoing a change therewith."

I think I can establish even more precisely the relations between single and multiple development and clarify them further by the following theories: The multiple invaginations of the gastrula, which serve as the starting point for the formation of multiple formations, show differences in their mutual behavior depending on the character of the egg and of the gastrulation process peculiar to it. These differences bestow a characteristic feature to the monstrosities in the individual classes of vertebrates.

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For illustration of this gastrulation theory of multiple formations I again make use of some diagrams. These are only supposed to show how the contrast which exists between the bony fishes and the birds in normal

development also appears again in the character of their malformations.

I begin with the bony fishes. In the diagram Fig. 11 (Plate 20) two invaginations have arisen on the edge of the germinal disk at a slight distance from one another and have formed two forward embryonic rudiments (K^1 and K^2) in that their invagination margins have folded up in a familiar manner in the direction of a radius. Rauber calls the smaller portion of the germinal disk margin [Z] which separates both embryos, the inner or medial interspace; the larger periphery he calls the outer or lateral interspace. Each of these divides again in this way, that the invagination continues for some distance on the germinal disk margin from the forward embryonic rudiment (or from the portion of the blastopore included in closure) into two segments, into the blastopore margin and into the overgrowth margin. Since among the teleosts gastrulation extends for a longer interval and thereby a rather considerable portion of the overgrowth the margin is transformed into blastopore margin, the smaller the distance between the two embryonic rudiments included in development, the earlier the inner interspace must be raised for enlargement of the blastopore margins extending from left and right. As a consequence of this the double gastrula cavities, which originally arose separately, must now run together toward the rear into a common hollow space. Diagram 12 has resulted from Diagram 11.

In the further course the blastopore margins can now enlarge at the expense of the overgrowth margin only more on the lateral interspace; they behave exactly like the margin portion of a simple gastrula and accordingly they fold up gradually in the medial plane to form a simple body portion, as represented in Diagram 13.

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It is obvious that the farther apart the first invaginations on the germinal disk margin are, the longer the two blastopore regions remain separated and the larger the two embryonic bodies, which arise by concrescence of the blastopore margins, will turn out. And in case the two blastopore regions lie more or less opposite one another on the germinal disk margin, they will generally be kept completely separated and, in that each closes to form a ring, will yield two completely formed embryos, which are connected only with a band of abdominal wall as a result of their common origin from one yolk. In this case too the overgrowth of the yolk must take place in a somewhat different manner than in the normal course, since the overgrowth margin is divided into two more or less symmetrical halves by the double embryonic rudiments. The overgrowth must become ring-shaped and push in between to separate both embryonic bodies.

In view of His and Rauber, that the entire germinal ring is embryonic margin and is raised to form the body, the explanation of "gastrodidymi or omphalodidymi" raises difficulties and demands some accessory assumptions. One should compare the presentation given by Rauber [50] in Virchow's Archiv (Volume 74, pages 78-81) in this regard. With my theory of numerous blastopore rudiments and on the basis of my grasp of the gastrulation process, every difficulty with the explanation ceases.

It will be of great interest now, from the viewpoint suggested, to contrast comparatively the bird, which we can generally regard as representative of amniotes, with the teleosts.

According to the analysis which was given on page 449, the gastrulation process in birds is one varying to a high degree. For in them only a small part of the germinal disk margin is transformed into blastopore margin. The latter closes mostly caudally to form a ring and is detached thereby from the overgrowth ring. A priori this must be of far-reaching influence on the formation of multiple formations. For if two invaginations arise on the germinal disk margin in the birds, then one can expect that they will be kept separated here, even with apposition, where they soon fuse into one in the bony fishes. For illustration I refer to the diagrams, Figures 11, 14, 15. Figure 11, which has already served us as a diagram for the initial position of two embryonic rudiments of a bony fish, is now supposed to represent a corresponding initial position for the double formation of a chicken. In consequence of the above emphasized conditions in the enlargement of the germinal disk a portion of the inner interspace now lasts here and only a slight portion of it serves for elongation of both primitive streaks by transformation in blastopore margin toward the left and right. Therefore (in contrast to the teleosts) up to their detachment these always remain separated from the germinal margin by a piece of interspace, which possesses the property of the overgrowth margin. While fusion of both blastopore regions is encountered in Diagram 12 and 13, in Diagram 14 and 15 permanent separation of these regions by their detachment from the germinal disk margin is encountered.

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What was theoretically developed here from the course of the gastrulation process is in fact confirmed by the observations which one has made about multiple formations in chickens and other birds and reptiles on many occasions. In fact in them multiple formations in typical fashion show a relation other than that in the bony fishes. If we look more closely through the descriptions and illustrations of Dareste [11], Panum [43] Rauber, Gerlach, Klaussner and others, then we find that very frequently within a common clear embryonic area there occur two or three embryos, separated from one another and more or less widely developed. Moreover, the heads are always directed toward the center of the clear germinal area, the tail ends toward the germinal disk margin as must be the case according to the law of their origin already emphasized by Rauber. The axis of the embryonic rudiments can show the most different angle of presentation to one another.

At times they are directed parallel, if they lie close beside one another, or they form an acute, or more often, an obtuse angle with one another. Finally they can even be so oriented that the axis of one falls into a direct extension of the other, the heads turned toward the center, the tail ends toward the outside. (Opposition position).

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It will be of interest to determine somewhat more precisely the differences between both vertebrate classes separately and to contrast them with one another.

In the bony fishes completely separated double formations, which connect only through the yolk sac, are very rare; in birds they form the majority.

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In the fishes the embryos only remain separated when they are laid down in opposition position, i.e. on opposite points of the germinal disk, for with each greater approximation, sooner or later fusion of their hind ends occurs. On the other hand in the birds, even with greater opposition we do not see fusion occur because the interspace remains for the most part as overgrowth margin. The multiple rudiments are therefore not only not brought near with their caudal ends but often they are even conveyed even further into divergent direction.

In the bony fishes double formations with secondarily fused head ends and double separated body and tail ends have never been observed; in birds they develop frequently since, as Gerlach [18] remarked, if the forward ends of the primitive streaks lie too close together toward the center of the germinal disk, "the conditions for a collision of the head ends of both embryos are preeminently present." Perhaps even as a consequence of the premature detachment of the blastopore margin from the germinal disk margin and of the growth of the hind portion of the primitive groove by intussusception, their forward ends are pushed together toward the center of the germinal disk even more as it corresponds to their first origin. "Accordingly, one finds", as Gerlach continued in his summary, "in double formations partly a more or less profound fusion of both heads, whereby these can even appear as a structure which is externally, to be sure, simple, but on the other hand is deformed to a high degree; partly one finds, however, only a more superficial connection of both heads, further of the neck and thoracic region."

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Finally, in the conversely irradiated embryonic rudiments the heads meet with one another directly in case of a coalescence, from which different forms of craniopathy result." Between the fusion of the head ends of two bird embryos and the formation of a single hind end in the double monster of a salmon there exists a fundamentally important difference. There it is a question of a secondary appearance together of already completely and normally established body sections, produced by lack of space, as a consequence of which the organs mutually encroach upon one another in their growth. Here, on the other hand, it is a question of the fusion of two body halves, which supplement each other to form a normal constituted body segment and as counterparts belong to one another.

While in the bony fishes malformations with forward doubling (single body with two heads) are most frequent, in birds they subside very much in number in relation to other forms. Rauber remarks about them: "Direct observations of forward divergence, i.e., Y-shaped double formations of chickens, pose difficulties for the explanation in a strange way. It is as if the conjunctive moment appearing so strongly in the further development of double rudiments of bony fishes can, under certain conditions, also appear in greater measure in double rudiments of birds than happens normally. Cases of this kind are relatively rare. Doubts can still exist about their

valuation and the necessity must therefore be emphasized of gathering further observations of this sort from earlier stages." Gerlach is of the view that these double formations of the chicken cannot be explained in the same manner as in the bony fishes, in spite of external similarity, and he has thereby become motivated to place a theory of bifurcation alongside the radiation theory of Rauber. According to Gerlach's explanation, in contrast to radiation bifurcation takes place in such a manner that "only a single embryonic rudiment radiates upon the area pellucida and this rudiment, in its further development, which is directed forward, sooner or later leaves the medial line in order to spread into two side pieces in a forking diverging manner. Each of the side pieces corresponds to the portion of the embryonic rudiment which is still not developed at the beginning of bifurcation, so that it develops doubly in front of the point of bifurcation while it appears single behind this point. The result of this is that the double malformations with forward duplication appear in life by bifurcation and their higher degree (Ischiopagus) presuppose a more premature appearance of bifurcation than those forms of forward doubling which concern only the head and face portions (Diprosopus, Dicephalus)." Now, however, there exists absolutely no basis for assuming the mode of formation of bifurcation for certain double malformations of birds and thereby assigning them an exceptional position, if we bear in mind the excellent presentation of Duval's [13] concerning the origin of the primitive furrow from the primitive streak. According to this, in the birds just as in the bony fishes, the blastopore margin is laid down on the germinal disk margin and is transformed by "conjunction" to form the primitive furrow. If, from this, two gastrula invaginations arise very close beside one another, two primitive furrows will have to result in the chicken, in the same manner as in the trout. The furrows are separated on the forward end for some distance and fuse into one toward the rear if the small inner interspace separating them is used to form the blastopore margin. In the birds this process presupposes only a much greater juxtaposition of both rudiments than in the bony fishes because of the changed relations between blastopore margin and overgrowth margin exhibited earlier.

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Finally, there are never found in fishes double monsters with axes directed parallel which are more or less widely fused in the plane of contact depending on the degree of approximation. In birds they are observed and they will result here when originally both gastrula invaginations lie only a little farther apart than in the case discussed previously. In these nevertheless rare occurrences, the inner interspace must possess such a size that it yields, upon extension of the germinal disk and upon simultaneous formation of both primitive furrows, only as much to these toward the left and right by transformation into blastopore margin as its overgrowth margin increases in extent at this time. Under these conditions the forming primitive streaks will always keep the same distance at their hind ends at different stages with the size of the inner interspace remaining the same while they must converge in the bony fishes with the disappearance of the interspace. Finally, in birds, if both gastrula invaginations lie still somewhat farther apart than in the last case, then the hind ends of the primitive furrows arising from them must diverge,

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because with a further enlargement of the inner interspace the overgrowth margin increases more in extent than the blastopore margin is formed and used for elongation of the primitive furrows.

In connection with my contrast of multiple formation by fish and birds attention should still be called to the fact that, as has already been emphasized by other parties (Raubert, Gerlach), "through the radiation theory a phenomenon frequently discussed on the part of teratologists finds its extremely simple and natural explanation: namely the fact which is at once striking in double malformations of a higher degree (complete axial duplication) that both components of them are connected with one another at analogous parts of the body" (Gerlach). Head with head, thorax with thorax must fuse with greater juxtaposition of both components, because these are oriented in the same direction preceding from the germinal margin.

The theory by which the different character of double malformations in bony fishes and birds has been explained can perhaps serve us still further for the explanation of why in vertebrates, which develop from small, holoblastic eggs, in cyclostomes, amphibians and ganoida double malformations have been observed either not at all or only very rarely.

A double embryo of the salamander, in which, however, the eggs are just exceedingly large, have been described by Braun [7]. Born [6] had the opportunity to collect several double embryos from a stock of RANA but did not publish more precise details of their anatomy. Meanwhile, if one considers how often frog spawn is investigated and the artificial fertilization of frogs' eggs carried out, then the scarcity of this kind of data must certainly surprise and make plain to us the assumption that in the animal classes named multiple formations probably will not be formed as frequently as in the teleosts and amniotes, and perhaps will not be formed at all. Even Raubert has emphasized this phenomenon and attempted to explain it by the fact that in holoblastic eggs "room for a second rudiment is very limited and that eggs of this character are extraordinarily little suited, not so much to establish double formations but much more to bring them to any advanced stage of development."

According to my view the phenomenon touched upon could be connected here also with the kind of gastrulation process, either in such a way that invaginations cannot form at all in two different places because of the limited space in the blastula or perhaps in such a way that two separate invaginations arise, to be sure, but fuse prematurely into one before it has come to coalescence of blastopore margins at two separate places and thereby to the formation of two separate medullary plates. In the latter case one could say that as regards the rudiment a multiple formation present in the embryo has, to a certain extent, remained latent because it had not found the possibility of its development.

Perhaps it can be hoped that, by investigation of frog's eggs at stages of development earlier than those which have served as the starting point of

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this work, an answer will be obtained to the question which has been posed. Also we might think about the fact that the frog's eggs described by me as arrest malformations perhaps stand in their extreme forms in a causal relation to multiple malformations and have been produced by the same conditions.

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A supposition of that kind cannot be completely rejected especially if one considers an observation by Rauber. According to him "restraints for the direct, further joining of the outer interspaces for the formation of a common body part easily appear with the presence of two forward embryonic rudiments. The dorsal fissure in the region of the common single body segment is then joined to form a forward doubling."

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However, we shall be able to obtain evidence regarding these and other questions only when we know beforehand what deeper causes must be active in the egg if, instead of a normal, single invagination, several separate gastrula invaginations are to develop or two embryos are to develop from a single egg cell.

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5. Under What Conditions Can Multiple Rudiments Arise From a Single Egg Cell?

The question posed in the previous chapter and serving in this chapter as a title naturally forces itself upon everyone who has concerned himself extensively with the study of multiple formations. In the literature there has not been a lack of hypotheses which are supposed to make the process more understandable for us. Experiments have also been frequently performed in order to produce malformations artificially by an experimental method, especially through mechanical injuries (splitting of the embryonic rudiment) or through other methods of attack. Whoever wishes to inform himself more closely in this regard is referred to the historical presentations by Rauber and Gerlach. It now lies within my power to show how, according to our present knowledge the answer to the question might turn out.

Different ways in which the original single rudiment of an egg cell can be increased to two or three appear to me to be possible. Such a way is the separation of the partial products of an egg into several parts which no longer influence each other mutually in their development.

According to the theory of heredity worked out more precisely by me in two publications [22] each component piece of the egg cell receives, through the process of nuclear division, just as much hereditary substance in its nucleus, both as to quantity and quality. Through the possession of this hereditary substance it carries within itself the possibility of reproducing the whole from itself under suitable conditions. I am glad to have obtained a new important argument for this conception in the interesting experiments of Driesch and Chabry.

JASA

According to a method already used by me for other purposes, Driesch [12] has shaken sea urchin eggs, after they have just divided for the first time, and has thus isolated in many cases both first cleavage cells from one another and has raised them further separately. In this way he obtained in 30 cases 30 normal blastulae of half size and in 15 cases, at the end of the second day, small gastrulae, from which three still developed further to form true Plutei. Driesch has herewith established the important fact which is in harmony with my conception of organic formation, that "an isolated cleavage cell, if it lives at all, always develops to form a structure which is distinguished from the normal only by its size," and that it produces from itself no half structure, in Roux's sense, but again "an entire individual of half size, a component structure." At times the egg membrane was not torn by the energetic shaking but "only strongly stretched and the contact of the cleavage cells, which is otherwise rather narrow in the two stage, became loosened." The result was that two small blastulae and two small gastrula developed in one egg membrane in several cases. Both cleavage cells arising by division from one egg had therefore yielded twins which were mostly completely separated from one another but stood in permanent union in one case.

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If it were possible in a frog egg divided into two half spheres, to remove one of them completely without any damage to the other, then from the component part a complete, normal only somewhat smaller frog larva would have to be grown. The component half would transform, after it had cleaved further, to form a normal blastoderm, a normal blastula, etc. in the same manner as the entire egg and would be reduced only in size as would become necessary through the halving of the substance capable of development. If it were further possible to shove an isolator, which abolished any relation between them, between the first two cleavage cells of a frog egg in its plane of contact, then an entire normal embryo, single and alone as a result of its isolation, would have to form from each half. From the egg twins

?

The results at which Roux [61] arrived in his treatise "on the artificial production of half embryos by destruction of one of the first two cleavage spheres," appear to harmonize only slightly with these conclusions. Roux himself has summarized his essential results briefly: "After destruction of one of the first two cleavage cells the other is able to develop in the normal manner to form an essentially normal half embryo. In this manner we obtained hemiembryones laterales and anteriores besides the corresponding first stages of semiblastula and semigastrula. Three quarter embryos with lack of a lateral head half were also obtained by puncture of the egg after the second cleavage."

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Driesch has already emphasized the contrast between his results and those obtained by Roux. I do not think now that this contrast really depends upon a different behavior of cleavage cells of an echinoderm and a frog, but is associated with deficiencies which accompany Roux's experiments.

Roux has not completely separated by his puncture experiments

the damaged cleavage cells from the process of development; he has damaged and retarded them only more or less in their development; moreover Roux himself has already felt this circumstance disturbing in his experiments. At first he only punctured the eggs, whereby extra ovata resulted; this procedure was soon given up, however, since even with repeated puncture with simple, fine needles and in spite of the large extra ovata thus produced, the cells developed normally. Therefore the needle was heated before the piercing, whereby better results were obtained; but even now these were still of the sort "that in some 20% of operated eggs only the uninjured cells survived the treatment, while the majority perished completely and some few, in which the needle had probably already been too cold, developed normally." Roux operated further with large masses of cells which were "not isolated" but lying together in balls after the formation of the first furrow, in order to select therefrom, after some hours or on another day, and to set up separately the ones in which the operated cleavage sphere had not furrowed.

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As I interpret the matter, Roux has therefore obtained no half blastula, half gastrula or a half embryo in his experiments. Instead he has obtained an entire blastula, an entire gastrula, an entire embryo which of course, as a result of the damage inflicted upon them, consisted of a normally and an abnormally developed component. In some of the cross-sections illustrated by Roux, the yolk mass was not divided into cells. That it was not eliminated from the development as a dead part could be concluded from the fact that it was permeated by numerous nuclei around which even radiation of the protoplasm had formed. Roux is inclined to derive the nuclei from the cleavage nucleus of the operated cleavage sphere, which therefore cannot have been killed by the treatment.

In the puncture experiments of Roux I see only one thing demonstrated, that in the undisturbed course of development the cell material of one side of the body is derived principally from one of the first two cleavage cells. As a result of the continuity of development, certainly in a natural manner, every older cell group must be able to be traced back to a preceding younger group and thus finally certain body portions must be able to be traced back to certain cleavage cells. On the other hand, I do not find the cardinal point demonstrated by Roux's experiments, that nothing else than the left body half must develop from the left cleavage cell under all circumstances, because it contained the differentiating and molding forces only for this.

The point of difference is of extraordinary significance for the valuation of organic developmental processes: according to Roux, the developmental processes are "not to be considered as a consequence of the cooperation of all parts or even of all nuclear portions of the egg, but in place of such differentiating reciprocal actions on one another, the self-differentiation of the first cleavage cells and of the complex of their derivatives advances to a certain piece of the embryo." Each of the first two cleavage spheres "thus contains not only the building material for the corresponding piece of the embryo, but also the differentiating and molding forces. The cleavage divides the portion of the germinal substance which

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accomplishes the direct development of the individual, especially of the nuclear material qualitatively, and determines simultaneously, along with the arrangement of these different, separate substances that occur, the position of the later differentiated organs of the embryo." Roux remarks, "Gastrulation takes place in each antimerie independently, and the same is also the case in the caudal and cephalic halves. Therefore, it is true also for the quarter concerned, and we can conclude with regard to the observed further development of this quarter; the development of the frog gastrula and of the embryo resulting therefrom is after the second cleavage, a mosaic process, and indeed results from at least four vertical, independently developing pieces."

I must place the antithesis, opposite of this thesis: 1) The development of an organism is no mosaic process; 2) The portions of an organism develop in relation to one another or the development of a part is dependent upon the development of the whole.

What this thesis, an antithesis finally amounts to, the reader will best learn if we consider the first two cleavage cells of the frog egg separated in the plane of division by an isolator, as was already mentioned above. Since no injurious treatment has taken place, aside from the suppression of interrelations, then development must proceed further on both sides of the isolator. Since each cleavage cell contains only the differentiating and molding forces for the left and right side of the body of the embryo, and since differentiating reciprocal actions are generally denied, according to Roux, at first a left and right blastula half, from each again a corresponding gastrula and finally two complete body halves would arise, which, if they were not separated in the medial plane from one another by the isolator, would be restored to form a normal embryo. According to my conception and that is probably also the conception to which Driesch will be led on the basis of his experiments, each of the two cleavage cells transforms into a cell mass from which a complete blastula, a complete gastrula, and a complete embryo of only moderate size arises. According to my conception therefore, each of the first two cleavage cells contains not only the differentiating and molding forces for a body half, but also for the entire organism, and the left cleavage cell develops in a normal manner to form the left body half only through the fact that it is placed in relation to a right cleavage cell. /481

I mentioned above as support for my conception, as well as Driesch's, the experiments of the French investigator Chabry [10]. In theory this investigator is of the same viewpoint as Roux. He assumes for the ascidians and for animals whose cleavage cells have differentiated early "that each blastomere contains in effect certain parts whose irremedial loss results in its death, and that the different parts of the animal are performed in the different parts of the egg." His experiments appear to me, however, to show just the opposite of that.

With the help of specially invented, ingenious instruments Chabry (1887)

has destroyed individual cleavage cells of *Ascidiella aspersa* by piercing with fine glass needles. If he now destroyed the left of the first two cleavage cells, which is supposed to contain the material for the left body half, then he should grow in several cases from the right, uninjured cleavage cell, a larva whose tail has the form, length and usual structure. The three layers of the blastoderm were distinct and the nervous system represented by a spot pigmented with small granules situated at the base of the tail in the concavity of its point of attachment to the trunk. In front of it there existed a papilla of fixation." If the right cleavage sphere was destroyed, then, as Chabry expressed it, he produced: left half individuals, of which he remarks: "They can show the same organs as the right half individuals with the exception, no doubt, of the otolith." The result was the same if the two right or the two left cells were destroyed at the second cleavage stage. Chabry illustrated one larva thus obtained with the remarks: "In spite of its striking resemblance to an ordinary larva, it is only, however, the moiety of a larva. The general form is good enough, the trunk and the tail are distinct." Even with destruction of the two cells situated to the front or to the rear or of only one of them, larvae could be grown. In the sense of his theory, Chabry lays stress upon the circumstance that upon destruction of certain cells, no otolith and only one adhesive papilla have been found. However, that is of very minor importance when one considers that all three germ layers, chord, muscles, nerve rudiments, intestinal canal, etc. as a whole have developed according to the norm, and probably the otolith and the adhesive papilla would also have arisen if the larvae, exposed to so many damaging manipulations (repeated study) had not died so early. In Ascidians, ocella are even formed in the region of artificially produced openings of their per-thoracic space (Loeb 39).

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At the conclusion of his treatise even Chabry himself tempers the full strength of his conception that the organs are preformed in individual cleavage cells by the sentence which one can consider as a half concession to my conception: "in effect it appeared to me that, by the death of a cell, the strength of the survivors was changed and that they then gave birth to those parts without which they would not have been produced."

The distinguished experiments by Chabry have, in my eyes, brought to light a very important conclusion, which Driesch has already summarized in the following sentences. "A half right or left embryo does not develop from the non-operated cleavage cell, but always an entire embryo of half size, which, of course, is lacking certain organs of lesser importance (otolith, and adhesive organ). The special experiments carried out and the pictures by Chabry make this certain: the result is thus opposed essentially to that of Roux."

The conception of organic development represented here harmonizes with the experiments of botanists and with the productive experiments of Loeb [39] extended in a similar manner to animal objects.

The way, discussed in the first place, in which multiple formations can originate from a simple egg appears to me not to have reference to any standard in the normal course of things. To divide the egg into two parts capable of development by mechanical action is no simple beginning. We can learn also by direct observation of bony fishes, that the multiple rudiments develop from an undivided germinal disk. "Fish eggs collectively, which harbor multiple formation at the neurula stage, were not larger than usual and were distinguished, aside from the multiple formation itself in being provided with nothing that wouldn't have been seen in single formation." From this Rauber concludes: "the embryo of a later multiple formation must already possess multiple design force, whose execution determines individuality, either before fertilization, which is more probable, or it must obtain it by the effect of the sperm on the egg. I believe also that it is a question of causes which must already have acted upon the egg before the cleavage process, and among the causes which come into question here, I maintain, that anomalies of the fertilization process (polyspermy or over-fertilization) are the most probable.

As was established first by myself [21, 27] and Fol [16, 17] in echinoderms, under normal conditions the egg is fertilized by a single spermatozoa, while in eggs which have suffered in some manner or another, more or less numerous spermatozoa penetrate. Only singly fertilized eggs develop normally, all other show disorders of the cleavage process and were therefore designated already in my first work as pathological. Fol has pursued the development of these further and found that blastoderms arise from them and that several invaginations form in these blastoderms. Therefore, he called them polygastrulae but could not keep them alive any longer beyond the stage indicated. He associated with them the hypothesis that double and multiple formations appear to result from multiply fertilized eggs.

Some years later Fol returned again to his hypothesis in a short communication in the Genfer Archive. He crippled the eggs of sea urchins temporarily by placing them in an atmosphere of carbon dioxide and thereupon produced polyspermy by the addition of sperm. He states that in this manner he obtained larvae which represent double and multiple malformations (polygastrulae). He modified his original hypothesis to the extent, as he allows, that sometimes the introduction of two spermatozoa produces no anomalous phenomenon. On the other hand, he believes that with the number 3 a limit is reached which cannot be exceeded by an egg without being punished. In this regard Fol remarks, "This fact has incontestable theoretical importance since it shows that the spermatozoon doesn't represent an individuality by itself, but only a certain dose of nuclear substance, without doubt possessed of special properties. The question of the origin of individuality is not absolutely linked to the dose nor to the source of the substance of the nucleus. The number of individualities which will take birth in a normal egg can only be recognized from the number of amphiasters which appear from the time of the first caryokinesis."

With shorter asphyxiation of the egg, according to Fol, three to four

spermatozoa penetrate into the egg and yield larvae which very frequently present double formations; with longer lasting asphyxiation polyspermy by 5-10 spermatozoa ensues. If further development occur (which is not always the case) manifold formations form (polygastrula monsters).

Independent of Fol my brother and I have crippled egg cells of echinoderms by chemical substances (especially narcotics), by mechanical shaking, by heat and cold and have produced slighter and higher degrees of polyspermy upon addition of sperm. We have extensively followed the developmental processes caused thereby. The original works [27] are to be examined with regard to very strange nuclear division figures, the bud cleavage, etc. However, we could obtain no assurance about the condition for the origin of multiple formations in the material for study. Of course the important fact appeared that multiply overfertilized eggs, in spite of the fact that they had divided in an entirely irregular manner into a mass of cells during the course of bud-cleavage finally yielded normal blastoderms in to to which, by virtue of their glittering, propelled themselves in a rotary manner in the water. Frequently, however, many did not develop further beyond this stage, in spite of the fact that they remained alive for some days, a phenomenon which also occurs very commonly with hybrid fertilization. Thus the eggs have obviously lost the life force, whether as the after effect of injurious treatments or through the act of polyspermy itself. With regard to the hypothesis of Fol, we have expressed ourselves as follows in the above mentioned work on the basis of our experiences:

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"During our stay at the sea, we have devoted special attention to the question of the origin of multiple formations. We have grown thousands of larvae from overfertilized eggs and studied the gastrula and pluteus stage since presumably at this time twin formation would have to be recognized best. But our output was diminishing to the vanishing point; we have found some few larvae with double gastrula invagination and some few plutei with double lips. The slight number of double gastrula amounting at most to about ten, is in no proportion to the thousands of single gastrula which we have grown from overfertilized eggs, so that one can utilize them in no way as proof for the view that double fertilization causes double malformations. Nevertheless, we would also not like to hold the theory as refuted thereby."

The latter is still my viewpoint even now. Although my experiments on frog eggs this time have likewise led to no positive results, I would still like to advise how to seek the solution still further in the way previously indicated. For one thing we can of course say even now with considerable certainty that an immediate, direct connection does not exist between overfertilization and multiple formation. It can no longer be thought that two spermatozoa cause a double formation by their penetration and that three cause a triple formation. Even Fol allows that in echinoderms, as Selenka has already emphasized, the introduction of two sperm nuclei produces no abnormal phenomenon. And my brother and I have obtained normal gastrulae and plutei from eggs of which a large part must have originated from

overfertilized eggs to judge from the relative proportions (individual cultures of overfertilized eggs were not carried out). Even now I have found that frog's eggs which showed completely irregular cleavage and therefore must have been multiply fertilized according to the general state of our knowledge, yielded normal larvae in individual cases in which they had been isolated. Just as normal individuals can be obtained from a minus amount of normal substance (from egg fragments or isolated cleavage spheres), normal individuals can also be obtained from a plus -- one egg and several spermatozoa. Thus we can contrast a partial development with an excess development.¹

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If even now no direct connection is demonstrable between overfertilization and multiple formation, such a connection could still exist in an indirect way. We must specially not overlook an important point. Every normally fertilized egg -- if I may borrow an expression of Chabry's -- "represents an organic system existing in balance in which it is impossible to disturb the position or form of one of its parts without having the other spontaneously and directly assume another state of equilibrium." (p. 147) Removal as well as addition of an equally organized part will produce a disorder under all circumstances as an interference with the system and regulatory processes will have to take place so that in spite of it, development approaches the norm. If disorders in the system are not compensated then they will be expressed in the first place chiefly as defects: artificial partial formations will easily show some sort of lack in this or any other place, as it results also in the experiments of Chabry. Shouldn't the opposite also occur under special circumstances with excess development?

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The penetration of two or more spermatozoa always causes a very considerable disorder in the process of development of the egg. If two sperm nuclei combine with the egg nucleus in Echinoderms, then a tetraster arises and instead of dividing in two, the egg is constructed equally into four cells at somewhat the same time, on penetration of three or more spermatozoa, the most complicated nuclear division figures appear² and the egg divides in the manner of a completely unregulated bud cleavage into a larger number of component parts which increase further in the manner of binary fission. If the protoplasmic body of the egg has not already been

¹ It seems to me to be not unthinkable and, to a certain extent, only a counterpart to the independent development of an egg half that two eggs, just fertilized, if they can be freed from their casing, joined together in the fashion of the first cleavage half spheres and surrounded by a common casing, would behave further in the fashion of a divided cleavage sphere and develop together to form a single embryo.

² Hearby I will remark, that in the number of chromosomes, which the daughter nuclei are allotted, very variable conditions must take place to which special attention will have to be directed in future investigations first to favorable objects, as well as to the question of whether in any manner a regulation of numerical proportions is produced in addition.

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damaged too much in its viability before fertilization, a group of small cells forms and these arrange themselves, in that they assume a cylindrical shape to form a blastoderm and one can no longer tell whether it is derived from a singly or multiply fertilized egg. Whether or not a multiple formation occurs can be decided only at the gastrula stage in that two or more invaginations become perceptible instead of one. We can therefore say: The disorder produced by overfertilization, after it has become latent for us in the blastoderm stage, finds expression again only in the alteration of the gastrulation process.

For echinoderm eggs Fol states that he has more frequently observed two gastrula invaginations; we have succeeded only in a few exceptional cases. According to these and other results, it will appear to me as if small, yolk-poor eggs are generally little suited for the production of multiple formations just because of their smallness, because on the blastula surface little space is present for multiple invaginations and the formation of an invagination could already be a basis for prevention of the formation of further ones. Rauber has already pointed to this moment in a somewhat different form. Therefore, asteracanthion eggs, which are larger than sea urchin eggs and possess more deutoplasm, which I likewise do not consider as unimportant, perhaps already offer more favorable conditions, as Fol has become mindful before anyone of the appearance of several gastrula invaginations in this object. /488

The size and the abundance of yolk of eggs seems to me to fall in the balance in the following manner. Let us take the meroblastic eggs of fishes and birds in which, up to now, multiple formation has been observed most frequently. With multiple fertilization many nuclear figures would form in the germinal disk which would thereby be more quickly divided into cells. Since nuclei accumulate protoplasm in their environment, then perhaps the protoplasmic parts also would be drawn hereby from the nutrient yolk toward the germinal disk even more so than already happens in a normal manner. The disk would therefore not only cleave more quickly but at the same time be even richer in substance capable of development and thus would be induced to production of two or three invaginations. However, multiple gastrulation serves as the starting point, demonstrable with certainty, for the typical multiple formations of vertebrates, as we have seen.

The question regarding the mode of action of overfertilization is complicated finally by still a further moment. Every healthy egg has a wonderfully arranged, regulatory ability through which overfertilization is prevented. That depends on a great irritability which brings about immediately contractible and secretory processes. From the stimulus produced by the contact with the head of an adequate spermatozoon the cell responds by shedding of a membrane and expressing of a slight amount of perivitelline liquor whereby the penetration of a further spermatozoon is made impossible. The entire mechanism works so promptly that artificially fertilized eggs of invertebrate animals, even if their surface is immediately covered by many spermatozoa upon mixing with a lot of seminal fluid, permit the penetration of only a single one. Therefore, overfertilization usually presupposes a

lesser degree of irritability of egg cells, a slower course of shedding of a membrane, and mainly a disorder of the regulatory mechanism; however, this will always be the result of lesser or greater injury which the egg has experienced before fertilization. As a result of this the disturbances will have to be added to the alterations of normal development caused by overfertilization. These disturbances are connected with the damage of the protoplasmic bodies of the egg cell present from the beginning.

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I discover a sign of a disturbance of this kind in overfertilized echinoderm eggs in the fact that from the egg content shining globules, to a certain extent dead parts which have become unused, are secreted into the cavity of the blastoderm (stereoblastulae). Many fundamental changes could be observed in overfertilized frog's eggs (Plate 20, Fig. 21 to 27). Sometimes only the animal half of the egg had entered the process of development (Fig. 21, 22), while the vegetative half, since it contained a lesser quantity of protoplasm, is not divided into cells and bears signs of disintegration (vacuolization, etc.). Nuclei and even individual cells (Z^1), isolated or lying together in groups, occur in it. Findings of this kind can only be interpreted to mean that in the damaged egg the viable substances and those which have died separate from one another as a result of fertilization, which is conveyed in a very different manner in the preparations illustrated in Table 20. In one egg (Fig. 23) islands of small, pigmented cells, spread out in one to two places, have formed only in the cortex of the animal pole on the vacuolized yolk mass; in another case (Fig. 21) a blastula has arisen in which only the animal half consists of cells while the vegetative half consists of partially dead yolk mass. And in some cases (Fig. 24, 26, 27) we see that the living substance, which has separated from the remainder of the egg (which is incapable of development) and often amounts to only one half or only a third of the whole, even invaginates to form a gastrula and even develops a nerve plate (Fig. 26, 27 mp) and chord. Thus partial formations have resulted which agree in many respects with the partial formations which Roux has produced by complete or partial destruction of one of the first two cleavage spheres.

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In the same series of phenomena I count the findings which Chabry [10] communicates to us about monstrous larvae of *Ascidella aspersa*. He states that in the fall toward the end of the spawning period and likewise also from individuals which have been in captivity for a longer time than he has obtained eggs which brought forth monsters almost exclusively. This showed early in irregularities of the cleavage process. Illustrations which Chabry has given of this agree with that which I have designated as bud cleavage in echinoderms. Furthermore, Chabry observed that parts of the egg died, at times half or even three fourths, and that there developed from the remainder which stayed viable monstrous larvae in which one could distinguish the three primitive germ layers and even different organs. Once a larva was also discovered which possessed two swimming tails and a double chord.

Although Chabry seeks to explain his findings only through the assumption of parents monstripores and through the doctrine of a monstrosity of the

germ cell, I still believe that in Ascidia it is also a question of damaged and multiply fertilized eggs, as Fol, Born [4], my brother and I have established in other objects. Page One Title

As it will have followed from my presentation, different factors must concur with one another in an overfertilized egg, forces which tend toward development and influences of a retarding and disturbing sort. The former are activated by fertilization, even if it is multiple; the latter are the results of damage which the egg has experienced before fertilization through overripeness and influences of a different sort. Accordingly as the first or the second factor predominates, the end results of development will have to turn out very differently.

Let us now survey from this viewpoint the teratological findings, which one has obtained from pike or salmon series. These it is shown according to the experiences of Lereboullet and Rauber that monstrous eggs occur abundantly in many stocks and offer different characters. Alongside typical multiple formations are found eggs with blastopore cleavage and among these again, the blastopore margin has developed at times only on one side to form a half embryo, while on the other side its organforming ability has been lost. Finally, we recognize eggs in which only the yolk has become overgrown by a thin cell layer, which no longer possess or possess only partially, the ability to produce an embryonic rudiment (Molen). In the latter case, the rudiment of a tail can have been established from the cell vesicle, while head and forward half of the body are lacking. Even in the monstrous frog eggs a series is offered to us: embryos with different degrees of blastopore cleavage, arrest of organ forming ability on one side of the blastopore margin, eggs in which only a partial development has taken place.

From what has gone before, the possibility should not be dismissed that the findings, which are so often of a different sort, stand in a causal association, that it is a question of eggs damaged in different measure and as a result of this, overfertilized, eggs in which the factors promoting the retarding process of development have asserted themselves in this way, in this or in that part of the rudiment.

One sees that the question regarding the connection between overfertilization and multiple formation is not as simple as it appears to be according to the hypothesis of Fol. It is a question of complicated circumstances which are still difficult to summarize in their connection. A relation between the number of penetrating spermatozoa and the kind of multiple formation is to be disregarded completely at first; on the other hand, the alterations of the normal course of the process of development produced by overfertilization, as well as the size and the organization of the egg in the formation of monstrosities will play a principal role, and will have to be considered above all in attempts at explanation. As dark as the entire field of monstrous development may still be, I feel still that a systematic, experimental investigation will succeed if it proceeds further in the direction whose way has been paved, to spread light over many things.

One will have to produce overfertilization by artificial methods in eggs of fishes, amphibians and invertebrate animals. Of these methods, those naturally deserve preference the most which damage the developmental ability of eggs the least (overripeness, treatment with carbon dioxide, cold, etc.). Abnormalities of the cleavage process will thereby be observed which become abnormally cleaved eggs, as Born [5] has already undertaken in a series of experiments, to be grown further in isolation. Success for these efforts will not be lacking with a choice of a suitable object.

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EXPLANATION OF FIGURES

a	anus	Cover Page Title
ab	optic vesicle	
ar	anal groove, anal tube	
af	anal folds	
ak	outer germ layer	
au	eye	
ch	chord, ch ¹ of the left side, ch ² of the right side	
chk	chord canal	
d	yolk	
dp	yolk plug	Inner Page Title
da	intestine	
dl	intestinal lip	
ds	intestinal bundle	
ed	hind-gut	
f	fin	
fh	cleavage cavity	
g	spinal ganglion	
h	adhesive disk	
hb	auditory vesicle	
hz	heart	
ik	inner germ layer	
k	head, k ¹ , k ² left and right head rudiment of the double formation	
kd	cephalic duct cavity	
ki	gills	
l	liver	
ls	left tail half	
mk	middle germ layer	
mr	medullary tube	
mp	medullary plate	
mw	medullary folds	
ms	muscle segment	

n	suture	
p	pigment	Page One Title
pr	primitive groove, pr ¹ , pr ² of the left and right rudiment	
r	body	Cover Page Title
rs	right tail half	
s	tail	
sf	tail fin	
sk	tail bud, sk ¹ , sk ² of the left and right side	
sch	septum	
t	infundibulum	
ud	primitive intestine	
ul	blastopore lip, ul ¹ , dorsal, ul ² , ventral	
uo	blastopore	Cover Page Title
ur	blastopore margin, ur ¹ , the part included in fusion, ur ² part situated in the germinal disk margin	
us	primitive segment	
ug	mesonephric duct	
uw	overgrowth margin (1-5 at different stages)	
va	vacuole	
vp	connection plate	
v	connection place	
z	interspace	
z ¹	cells in the yolk	
z ²	cells divided by furrows on the surface	
	entrance into the cephalic duct cavity	

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Explanation of Figures in Tables 16 - 20

w - female
get - killed
befr- fertilized
cons- preserved

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Plate 16
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- Fig. 1 Malformed Frog Embryo (Bm) With the Highest Degree of Blastopore Cleavage, Seen From the Dorsum. Egg of a Female Frog Killed on March 14, Fertilized on the 16th, and Preserved on the 23d.
- Fig. 2 A Similar Malformation Bf seen from the Dorsum. Egg Fertilized on the 17th, Preserved on the 23d.
- Fig. 3 Lateral View of Fig. 2
- Fig. 4 A Similar Malformation Bb
- Fig. 5 A Similar Malformation Ba
- Fig. 6 Malformation J¹
- Fig. 7 Malformation J³. Embryos J¹ and J³ are Derived From Eggs of a Female Killed on the 14th. They Were Fertilized on the 16th, and Preserved on the 21st.
- Fig. 8 Already Rather Widely Developed Malformation Bk With Normally Formed Head End (Optic and Auditory Vesicles) With Blastopore Cleavage in the Middle of the Dorsum in Front of the Tail End Which is Turned Back Dorsally.
- Fig. 9 Malformation N With Closure of the Blastopore in the Most Forward Segment of the Body, While it is Otherwise Still Open to a Complete Extent. The Embryo is Derived From the Egg of a Female Killed on the 14th. The Egg Was Fertilized on the 16th and Killed on the 23d.
- Fig. 10 Malformation M. Female Killed on the 14th, Fertilized on the 16th, Preserved on the 23d.
- Fig. 11 & 12 Malformation O With Partially Developed Head End, Extensive Blastopore Cleavage and Double Tail Bud. Fig. 11, View From the Tail Side in Order to Show the Surface. Fig. 12, Embryo Seen Somewhat Laterally From the Dorsal Surface. (W, 14th Killed, 16th Fertilized, 23d Preserved.)
- Fig. 13 Malformation F With Normally Developed Head End, Blastopore Cleavage in the Middle of the Dorsum and Appearance of Tail Buds. (W, 14th Killed, 16th Fertilized, 23d Preserved).

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- Fig. 14 Malformation G With Normally Developed Head End and Forward Body End, With Blastopore Cleavage in Front of the Tail Root (W, 14th Killed, 16th Fertilized, 23d Preserved).
- Fig. 15 & 16 Cover Page Title
Malformation With Blastopore Cleavage and Dwarfing of the Right Side of the Body, With a Developed Left and Tail Half. (W, 17th Fertilized, 23d Preserved.)
- Fig. 17 Malformation T With Closed Head and Body, But Split Tail End. (17th Fertilized, 23d Preserved)
- Fig. 18 & 19 Malformation Bg With Little Developed Head End With Split Tail End, With Wide Blastopore Margin, Fig. 18 With Lateral View, Fig. 19 With View From the Dorsum.
- Fig. 20 Widely Developed Embryo S With the Remainder of a Blastopore Cleavage in Front of the Tail End Which is Turned Back Dorsally. (17th Fertilized, 23d Preserved)
- Fig. 21 Widely Developed Embryo B With the Remainder of a Blastopore Cleavage in Front of the Tail End Which is Turned Back at a Right Angle.
- Fig. 22 Widely Developed Embryo V With Tail End Turned Back at a Right Angle, A Small, Hardly Visible Opening on the Dorsum and the Remainder of a Blastopore Cleavage. (17th Fertilized, 23d Preserved)
- Fig. 23 Malformation P Similar in Shape to Fig. 22. (W, 14th Killed, 16th Fertilized, 25th Preserved)
- Fig. 24 Pike Embryo at the End of the Third Day With Blastopore Cleavage.
- Fig. 25 The Same on the Seventh Day. Copy According to Lereboullet. Both Figures are Reduced by One Half and Have Been Drawn With Indian Ink.
- Fig. 26 Malformation Cb. In the Head and Body Region, In Which the Blastopore Has Closed, the Medullary Folds Develop, But On The Hind End the Blastopore is Still Wide Open. (17th Fertilized, 21st Preserved)
- Fig. 27 Malformation Ca. Egg In Which the Medullary Folds Develop at the Head End, While the Hind End Shows a Blastopore Still Wide Open. The Malformation, Descended From Eggs of a Female Killed on the 14th, Which Were Fertilized on the 16th.

The Egg Showed Irregular Cleavage and Was Isolated, Preserved on the 23d.

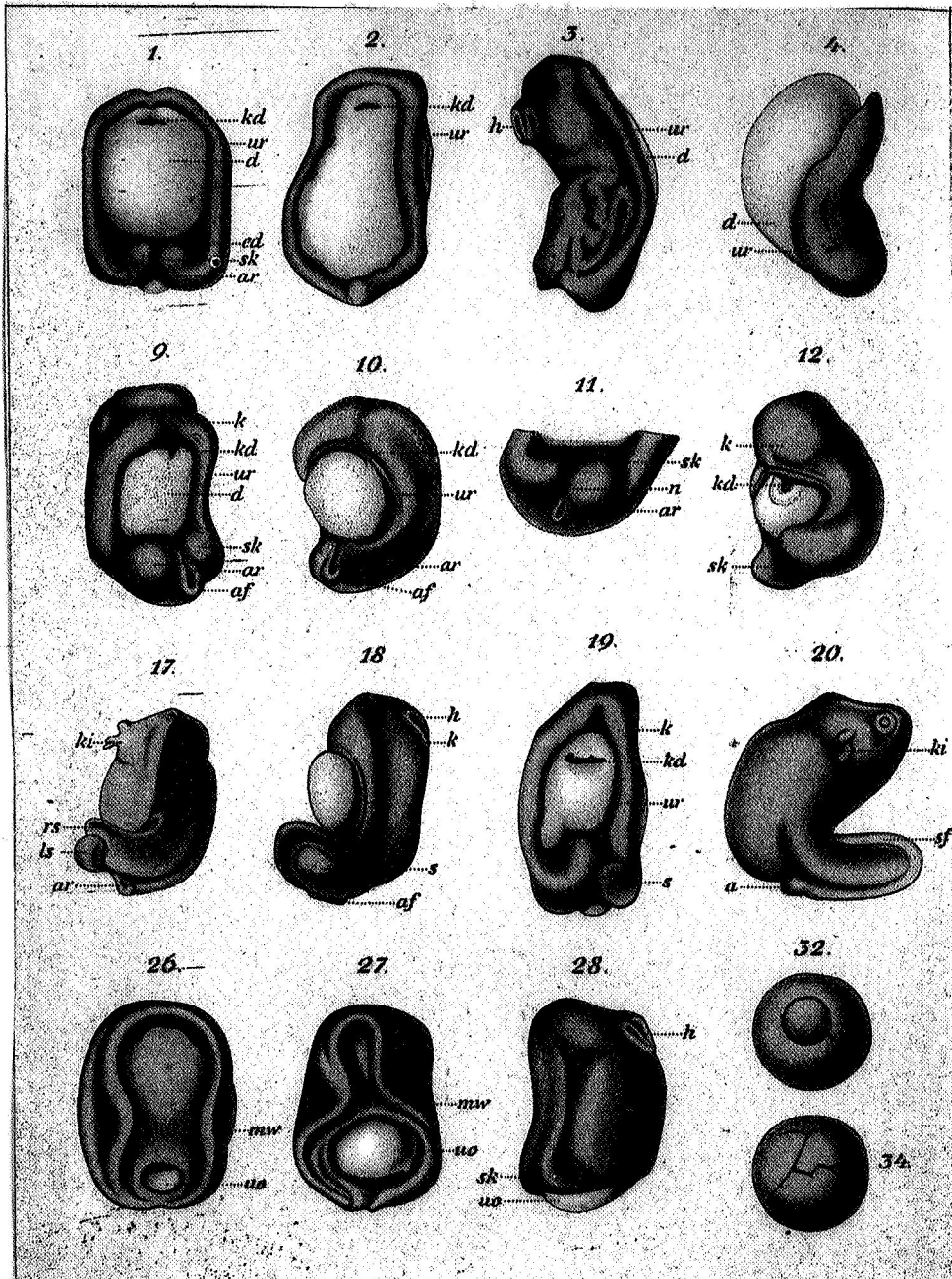
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- Fig. 28 Malformation Ad With Normally Developed Dorsal Region, But a Wide Blastopore Cleavage on the Hind End Beneath the Tail Bud, Seen From the Side.
- Fig. 29 Malformation Ac With the Remainder of a Blastopore Cleavage on the Hind End, Seen From the Dorsum.
- Fig. 30 Malformation Ab With the Remainder of a Blastopore Cleavage On the Hind End, Where it is Pushed Somewhat to the Ventral Side, Seen From the Ventral Side.
- Fig. 31 Malformation H With Beginning Tail Formation and the Remainder of a Blastopore Cleavage, Which is Found Ventrally In Front of the Tail Root, Seen From the Side. (S, 14th Killed, 16th Fertilized, 23d Preserved)
- Fig. 32-35 Eggs of a Female Killed on the 14th, Which Was Fertilized on the 16th and 4 Hours After Addition of Sperm Showed Baroque Cleavage in the Manner Sketched. The Eggs Were Isolated. From the Eggs Illustrated in Fig. 22 and 23 There Developed Embryos With Medullary Folds, In the Eggs Illustrated in Fig. 34 and 35 No Gastrulation, etc. Appeared.

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Plate 16
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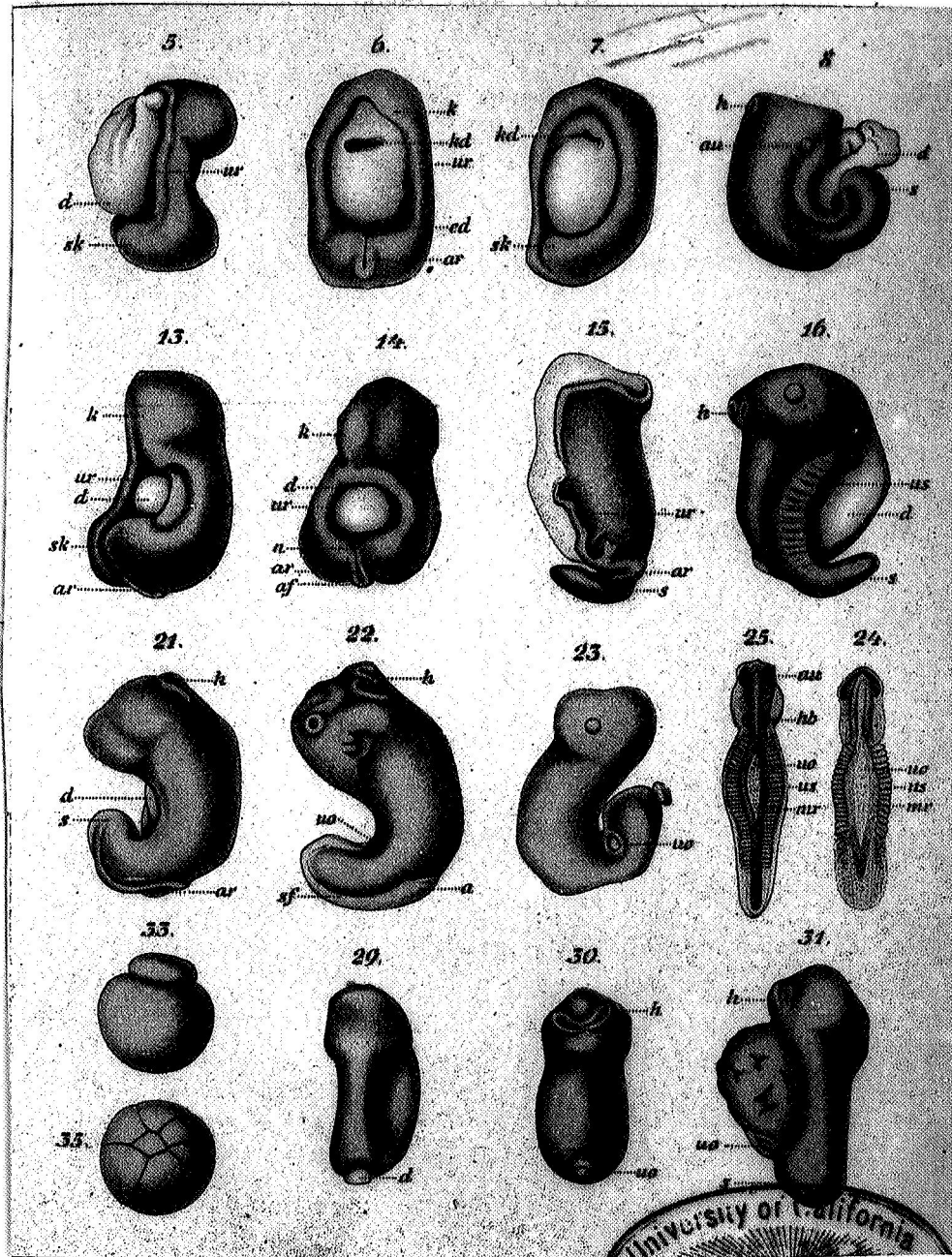


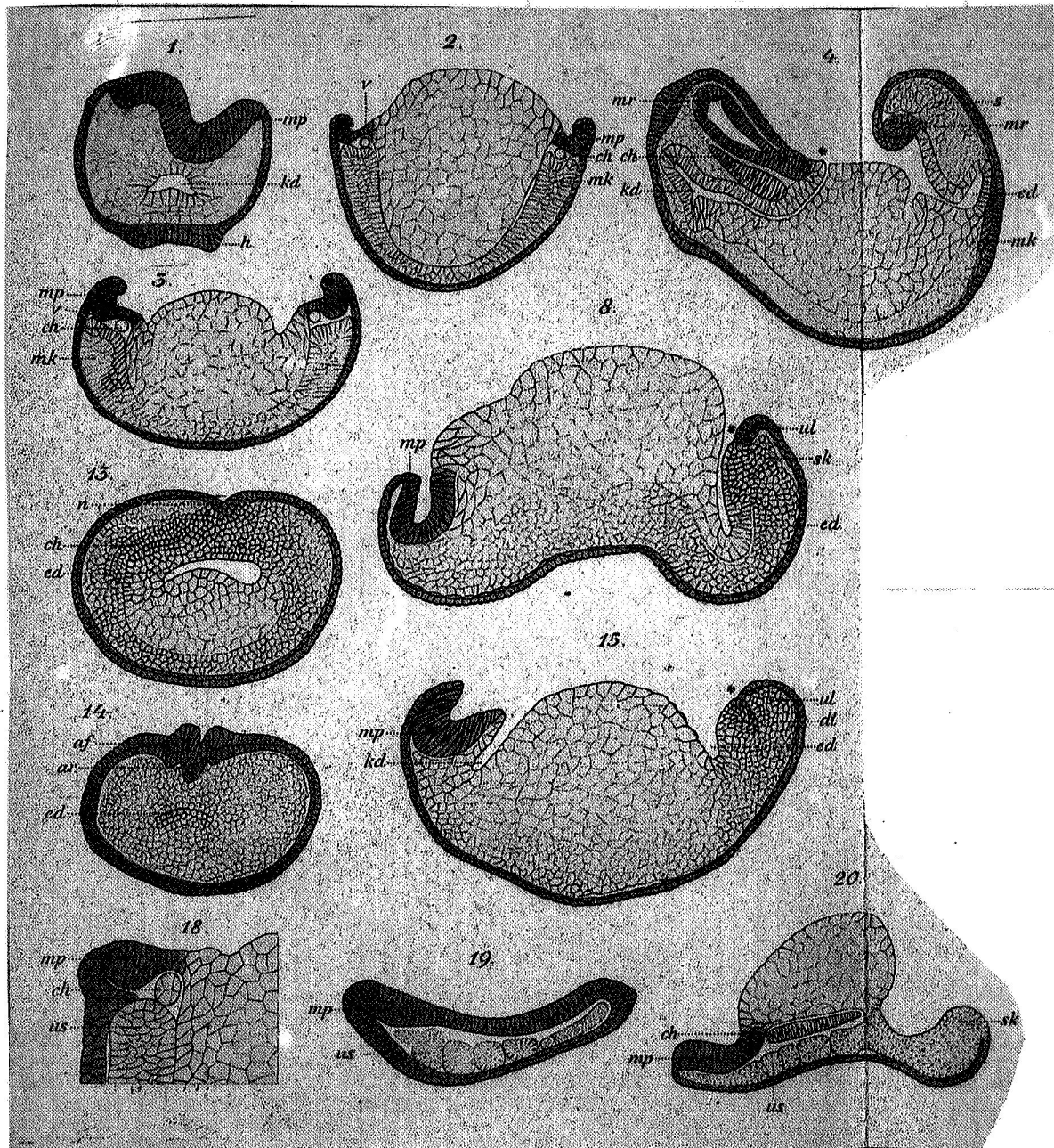
Plate 17

- Fig. 1 Cross-section Through the Foremost Head End of Malformation J¹ (Plate 16, Fig. 6).
- Fig. 2 Cross-section Through the Middle of the Body of the Same (J¹).
- Fig. 3 Cross-section Through the Hind Third of the Body of the Same Malformation (J¹).
- Fig. 4 Sagittal Section Near the Medial Plane Through Malformation O (Plate 16, Fig. 12).
- Fig. 5 From the Same Object A Sagittal Section Which Rather Coincides With the Medial Plane and From Which Only the Tail End Has Formed.
- Fig. 6 Cross-section Through the Lateral Blastopore Margin of the Same Object Somewhat in the Middle of the Body.
- Fig. 7 Cross-section Through the Portion of the Lateral Blastopore Margin of Malformation F Which Hasn't Reached Closure (Plate 16, Fig. 13). See Also Plate 18, Fig. 19-25.
- Fig. 8 Medial Section Through Malformation Ba (Plate 16, Fig. 5). See Also Fig. 11, 12 and 20 of Plate 17.
- Fig. 9 Medial Section Through Malformation M (Plate 16, Fig. 10). See Also Fig. 17, Plate 17.
- Fig. 10 Cross-section Through the Lateral Blastopore Margin in the Hind Third of the Body of Embryo Bf (Plate 16, Fig. 2). See Also Fig. 16 and 22, Plate 17.
- Fig. 11 Cross-section Through the Lateral Blastopore Margin in the Forward Third of the Body of Malformation Ba. See Also Fig. 8 and 12.
- Fig. 12 From the Same Object a Cross-section Through the Lateral Blastopore Margin in the Hind Third of the Body.
- Fig. 13 Cross-section Through the Hind End of Malformation J¹ (Plate 16, Fig. 6) at the Plate Where the Blastopore Margins Have Folded Up Over the Hind Gut Cavity to Form the Tail Suture (See Also Plate 17, Fig. 1-3).

- Fig. 14 The Same, Still Somewhat Further to the Rear.
- Fig. 15 Medial Section Through Malformation J³ (Plate 16, Fig. 7).
See Also Figures 18, 19, and 21 in Plate 17..
- Fig. 16 Medial Section Through Malformation Bf (Plate 16, Fig. 2).
See Also Figures 10, 22 in Plate 17.
- Fig. 17 Sagittal Section at Some Distance From the Medial Plane of
Malformation M (Plate 16, Fig. 10). Compare also the Medial
Section Fig. 9.
- Fig. 18 Cross-section Through the Lateral Blastopore Margin of
Malformation J³ From Which Sagittal Sections Are Illustrated
in Fig. 15, 19, 21.
- Fig. 19 Sagittal Section Through the Medullary Plate and the Primitive
Segments on the Lateral Blastopore Margin of the Same Mal-
formation (See Fig. 15 and 18).
- Fig. 20 Sagittal Section in the Region of the Chord Situated On the
Lateral Blastopore Margin of Malformation Ba (See Also
Fig. 8, 11, 12).
- Fig. 21 Sagittal Section in the Region of the Chord Situated on the
Lateral Blastopore Margin of Malformation J³. Preparations
of the Same Series of Sections Are Also Illustrated in Fig.
15 and 19. (Compare Also the Cross-section Fig. 18).
- Fig. 22 Cross-section Through the Lateral Blastopore in the Forward
Third of the Body of Malformation Bf. (Compare Also the
Cross-section Fig. 15 and the Medial Section Fig. 16).

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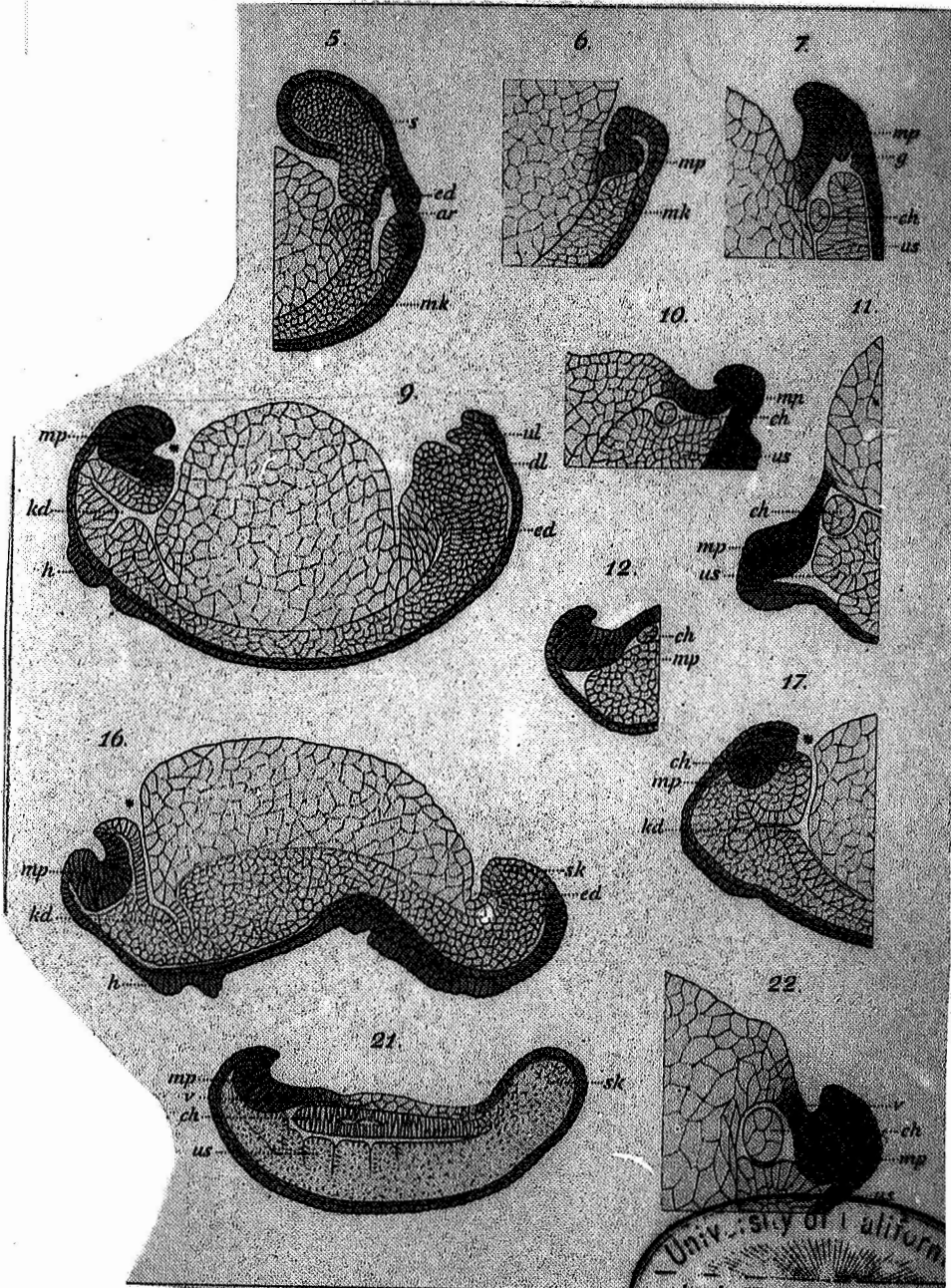
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Plate 18

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- Fig. 1 Cross-section Through the Head End of Malformation N (Plate 16, Fig. 9).
- Fig. 2 From the Same Object A Cross-section of the Series Following Somewhat More Toward the Rear in Which Left and Right Brain Halves Withdraw From One Another.
- Fig. 3 From the Same Object a Cross-section Through the Forward Third of the Body in the Region of the Blastopore Cleavage.
- Fig. 4 From Fig. 3 the Left Blastopore Margin Drawn at Somewhat Greater Magnification.
- Fig. 5 Cross-section Through the Hind End of the Same Malformation N, In the Region of Fusion of Left and Right Blastopore Margin.
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- Fig. 6 A Cross-section of the Series Following Somewhat Further Toward the Rear of the Same Object.
- Fig. 7 A Cross-section Through the Anal Groove of the Same Object, Somewhat More Greatly Enlarged.
- Fig. 8 A Horizontal Section Through Malformation W Through the Hind End in the Region Above the Anal Groove.
- Fig. 9 A Horizontal Section of the Series Following Somewhat More Ventrally Through the Anal Groove of the Same.
- Fig. 10-12 Three Horizontal Sections of a Series Through the Hind End of Embryo G. (Plate 16, Fig. 14). Fig. 10 Section Above the Anal Groove, Fig. 11 Section Through the Anal Groove. Fig. 12 Section Beneath the Anal Groove.
- Fig. 13-15 Individual Cross-sections From a Series Through the Curved Back Tail End of Malformation Bk (Plate 16, Fig. 8).
- Fig. 16 Cross-section Through the Hind Head Portion of Malformation Bg (Plate 16, Fig. 18) From the Region Where the Brain Halves Withdraw Laterally From One Another End the Chord Divides.
- Fig. 17 From the Same Embryo a Cross-section Which Follows Soon From the Foremost Region of the Blastopore Cleavage Where the Cephalic Duct Cavity Opens to the Outside. See Also Fig. 26.

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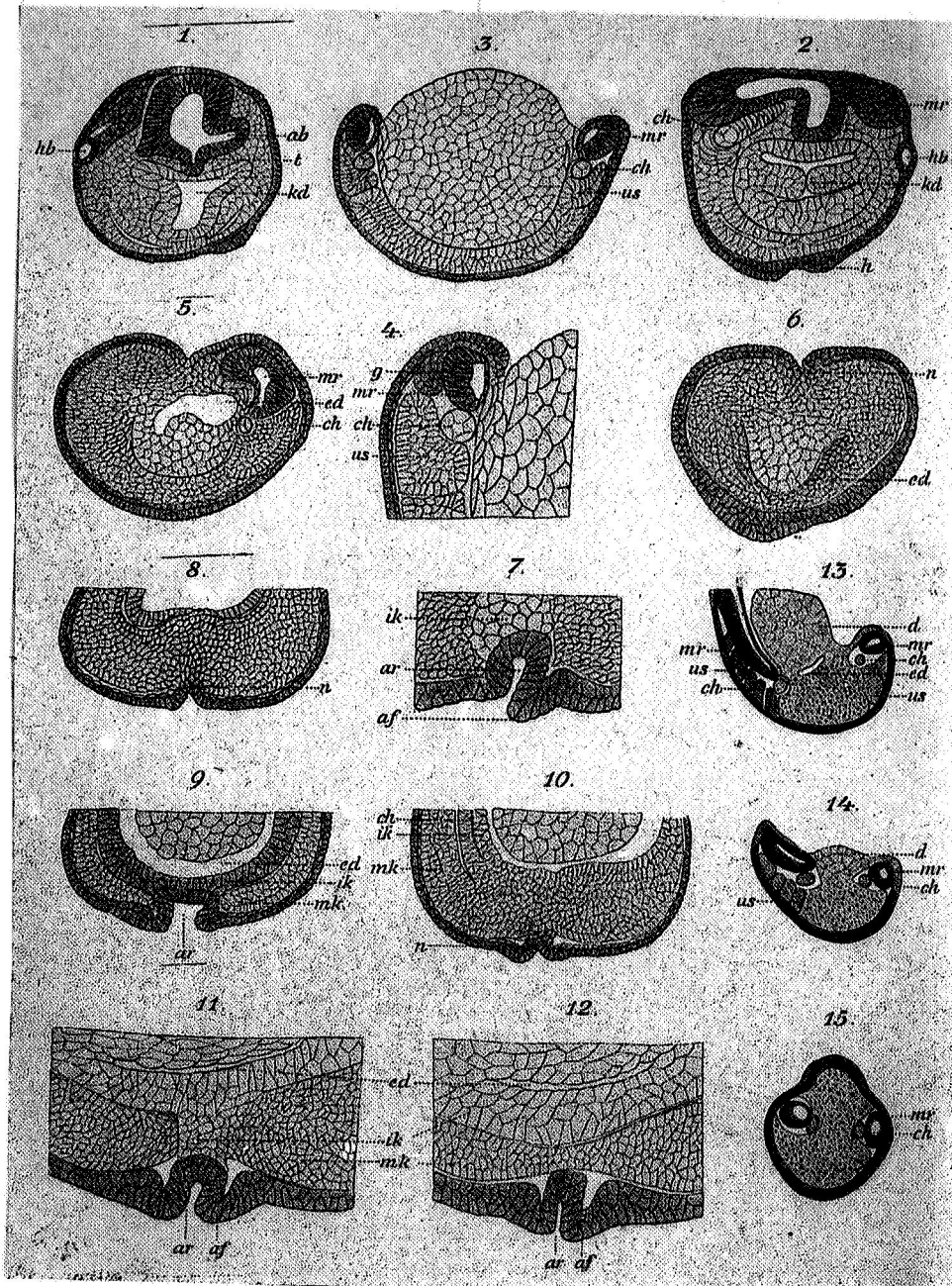
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- Fig. 18 Cross-section Through Embryo F (Plate 16, Fig. 13) From the Region Behind the Head Where the Division of Spinal Cord and Chord Begins.
- Fig. 19-21 From the Same Malformation Consecutive Cross-sections of the Series. Fig. 21 Section Through the Orifice of the Cephalic Duct Cavity. See Also Plate 17, Fig. 7.
- Fig. 22-25 Frontal Sections From a Series Through the Hind Body End of Malformation F, Whose Forward Body Portion Was Analyzed in Cross-section (Fig. 18-21). Fig. 22 Cross-section Through the Hind End of the Blastopore Cleavage.
- Fig. 23 Cross-section Through the Place of Fusion of the Rear Blastopore Margins.
- Fig. 24 Cross-section Through the Tail Root and the Anal Groove.
- Fig. 25 Cross-section Through the Tail Bud.
- Fig. 26 Cross-section Through the Cruved Back Tail Rudiment, Split Into Two Halves, of Malformation Bg (Plate 16, Fig. 18 and 19). Se Also Fig. 16 and 17 (Plate 18).
- Fig. 27 Cross-section Through the Middle of the Body of Malformation X (Plate 16, Fig. 15 and 16), With Right Blastopore Margin Curved Forward.
- Fig. 28 Cross-section Through Malformation H (Plate 16, Fig. 31) In the Region of the Remainder of a Blastopore Cleavage Situated Ventrally in Front of the Tail Root.
- Fig. 29 Copy According to Oellacher (i.e. Plate 18, Fig. 6). Reduced by One Half. Cross-section Through a Katadidymus of Trutta Fario Which Possessed a Blastopore Cleavage Up To the Lobus Opticus. The Section is Made Through the Liver Region.
- Fig. 30. Copy According to Lereboullet (i.e., Fig. 32). Germinal Fold of a Pike Egg 50 Hours Old. Yolk Hole. Germinal Fold. Extension of It.

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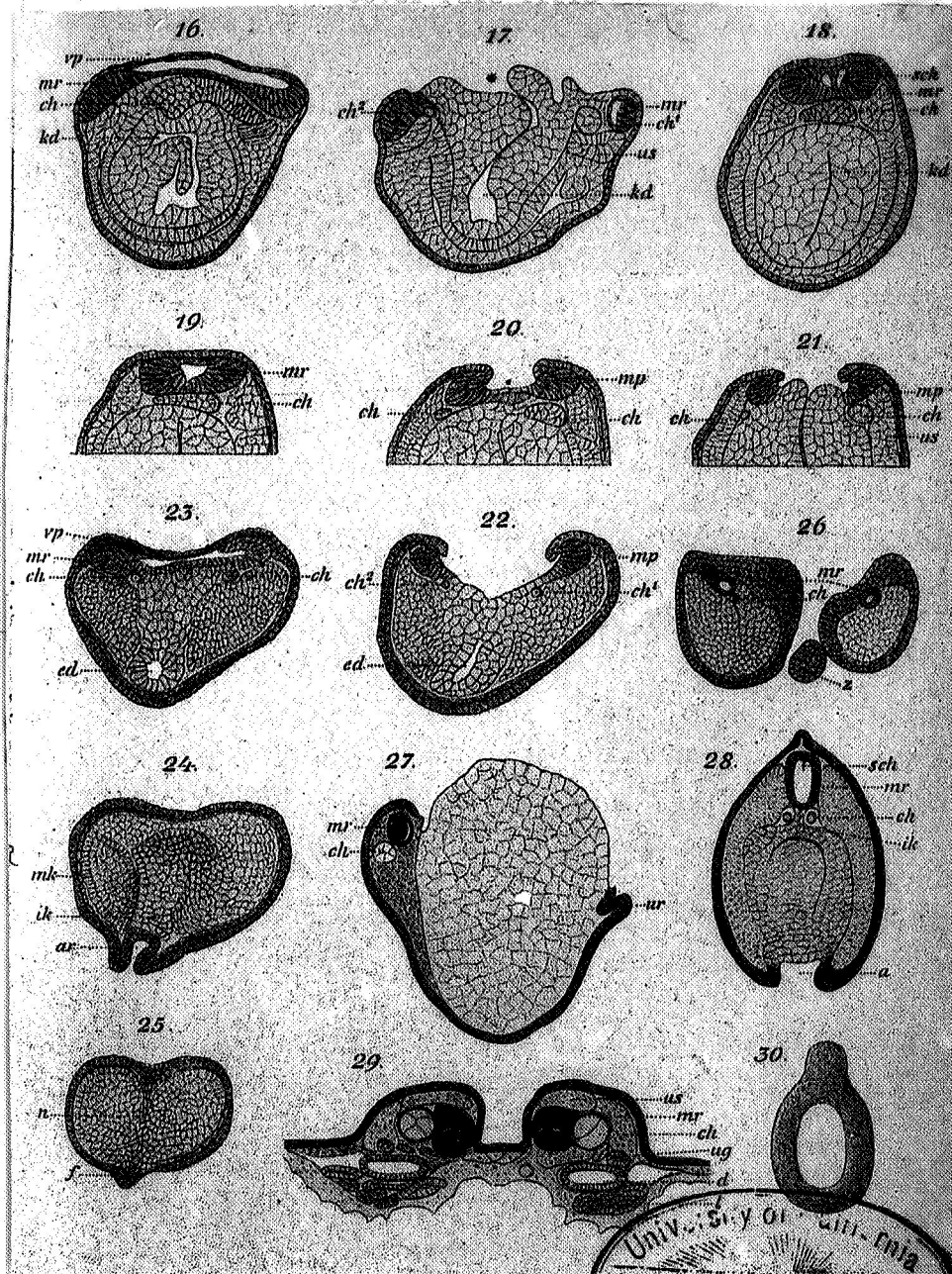


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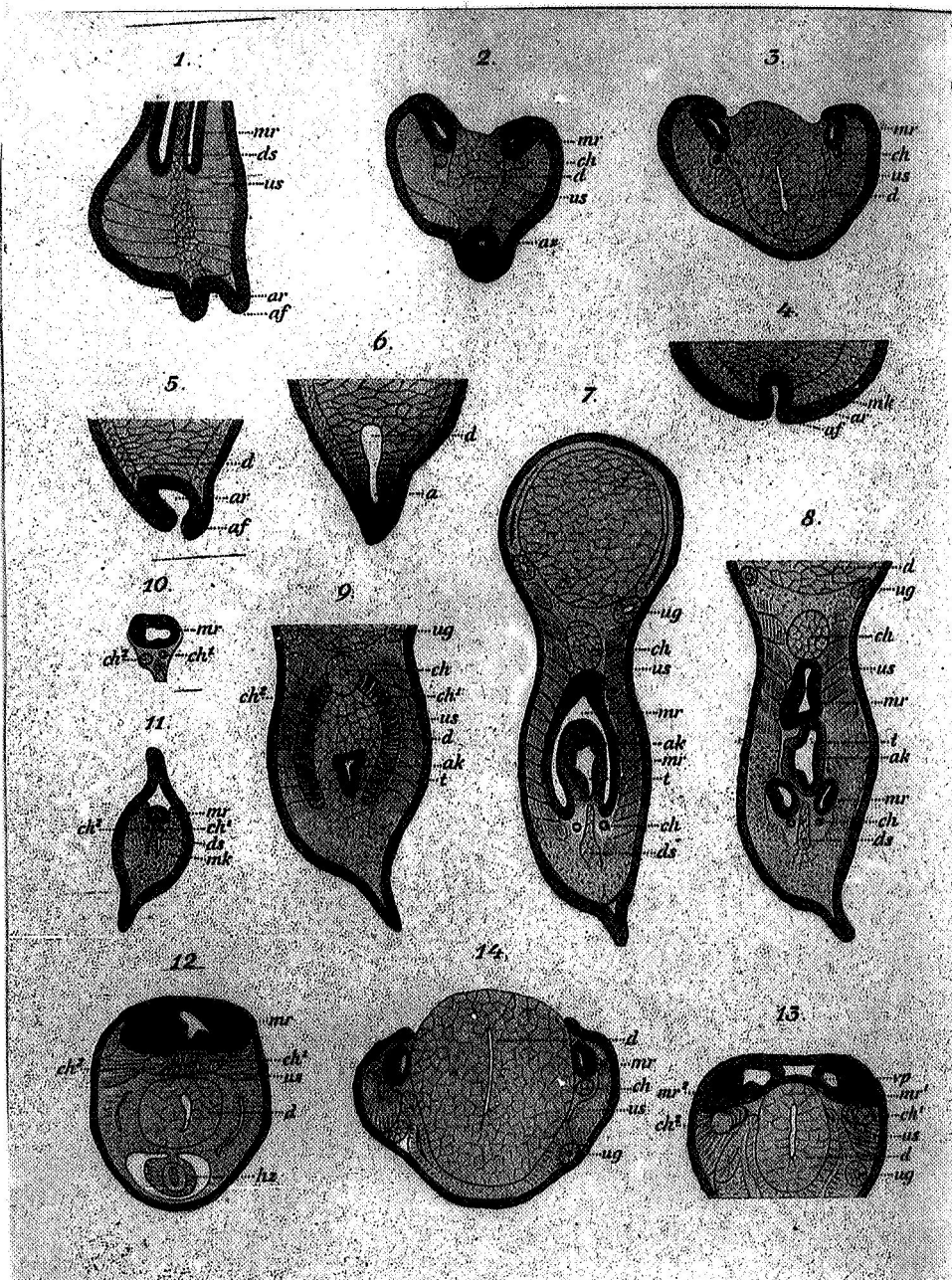
Plate 19

- Fig. 1-3 Cross-section Through the Tail End of Embryo B (Plate 16, Fig. 21) With Anal Groove.
- Fig. 4 Cross-section Through the Anal Region of Embryo K.
- Fig. 5 & 6 The Same of Embryo V (Plate 16, Fig. 22).
- Fig. 7-9 Three Horizontal Sections Through the Body of Embryo V (Plate 16, Fig. 22). Fig. 7 Through the Split Spinal Cord. Fig. 9 Through the Split Chord. Fig. 8 Through the Opening Of the Blastopore Cleavage (t) On the Dorsum.
- Fig. 10 & 11 Cross-section Through the Tail End of Embryo V (Plate 16, Fig. 22) With Doubled Spinal Cord and Doubled Chord (ch^1 , ch^2).
- Fig. 12-14 Three Cross-sections Through Embryo A. Fig. 12 In the Region of the Occiput. Fig. 13 At the Place of Splitting of Spinal Cord and Chord. Fig. 14 Through the Blastopore Cleavage.
- Fig. 15 Horizontal Section Through Embryo P (Plate 16, Fig. 23) At the Opening of the Blastopore Cleavage.
- Fig. 16 Cross-section Through the Tail End of the Same Embryo.
- Fig. 17 Longitudinal Section Through Embryo Ad (Plate 16, Fig. 28).
- Fig. 18-20 From a Series of Sections Through the Tail End of Embryo C With Splitting of the Chord (ch^1 , ch^2).
- Fig. 21 Horizontal Section Through Embryo T (Plate 16, Fig. 17 With Double Tail (s^2 , s^1)).
- Fig. 22 & 23 Two Horizontal Sections Through Embryo S (Plate 16, Fig. 20). Fig. 22 Through the Split Spinal Cord. Fig. 23 Through the Split Chord.
- Fig. 24 Cross-section Through the Tail End of the Same Embryo.
- Fig. 25 Cross-section Through the Anal Groove of the Same Embryo.
- Fig. 26 Medial Section Through Embryo Ab (Plate 16, Fig. 30).
- Fig. 27 Cross-section Through Embryo Ca (Plate 16, Fig. 27) At the Blastopore Cleavage.

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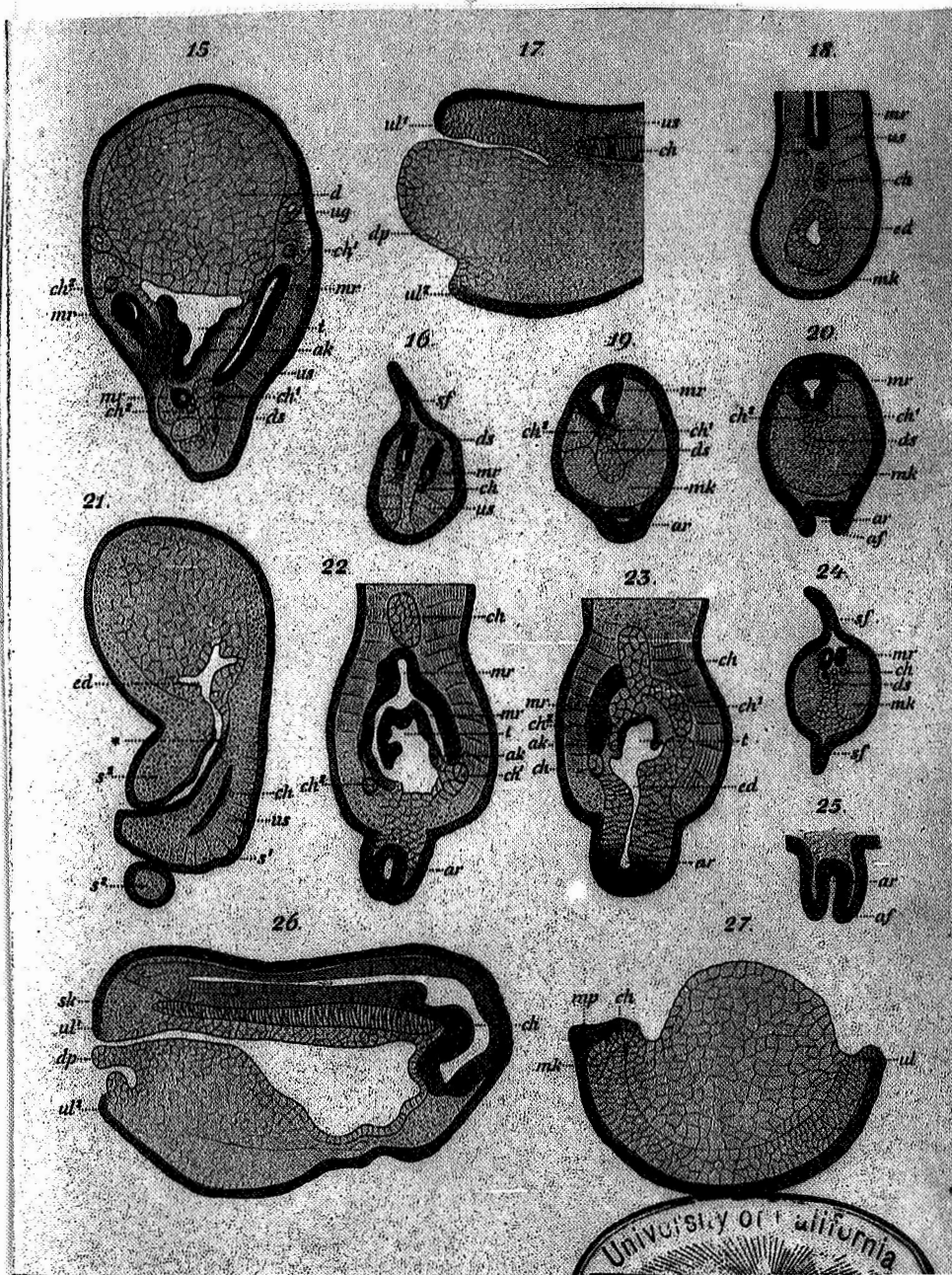
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- Fig. 1-4 Diagrams to Show The Formation Of a Salmon Embryo Through Drawing Together and Coalescence of the Blastopore Margins and the Relation of the Blastopore Margin (ur) to the Overgrowth Margin (uw).
- Fig. 5 & 6 Diagrams to Illustrate the Formation of Blastopore Cleavage Through Defective Closure of the Blastopore Margins (Arrest) in Salmon Embryos.
- Fig. 7 & 8 Diagrams to Explain the Condition of Blastopore Margin (ur) and Overgrowth Margin (uw) in Sea Urchin Embryos.
- Fig. 9 & 10 Diagrams to Explain the Same Condition as in Fig. 7 and 8 For the Eggs of Birds and Reptiles.
- Fig. 11-13 Diagrams Regarding the Formation of Double Malformations of the Salmon From Two Gastrula Invaginations.
- Fig. 14 & 15 Diagrams for Explanation of Double Malformation of the Chicken.
- Fig. 16 Diagrams For Explanation of the Chord Canal in Mammals.
- Fig. 17-20 Four Diagrams to Compare the Arrest Malformations With Blastopore Cleavage Fig. 17, 18 With Normal Development Fig. 19, 20.
- Fig. 21-23 Pathological Eggs In Which Cell Formation Has Appeared Only In a Limited Area, While the Greatest Part of the Yolk Has Not Divided.
- Fig. 24 Partially Developed Egg In Which the Segment Divided Into Cells Has Formed Two Germ Layers and Had Transformed to a Sort of Gastrula.
- Fig. 25 An Isolated Cell (Z) Surrounded by Individual Yolk Mass. A More Strongly Enlarged Part of Fig. 21.
- Fig. 26 & 27 Two Eggs In Which Half of the Yolk Has Not Developed, The Other Half Is Divided Into Cells, Which Have Been Arranged To Form Two or Three Germ Layers and Have Even Laid Down a Medullary Plate (mp).

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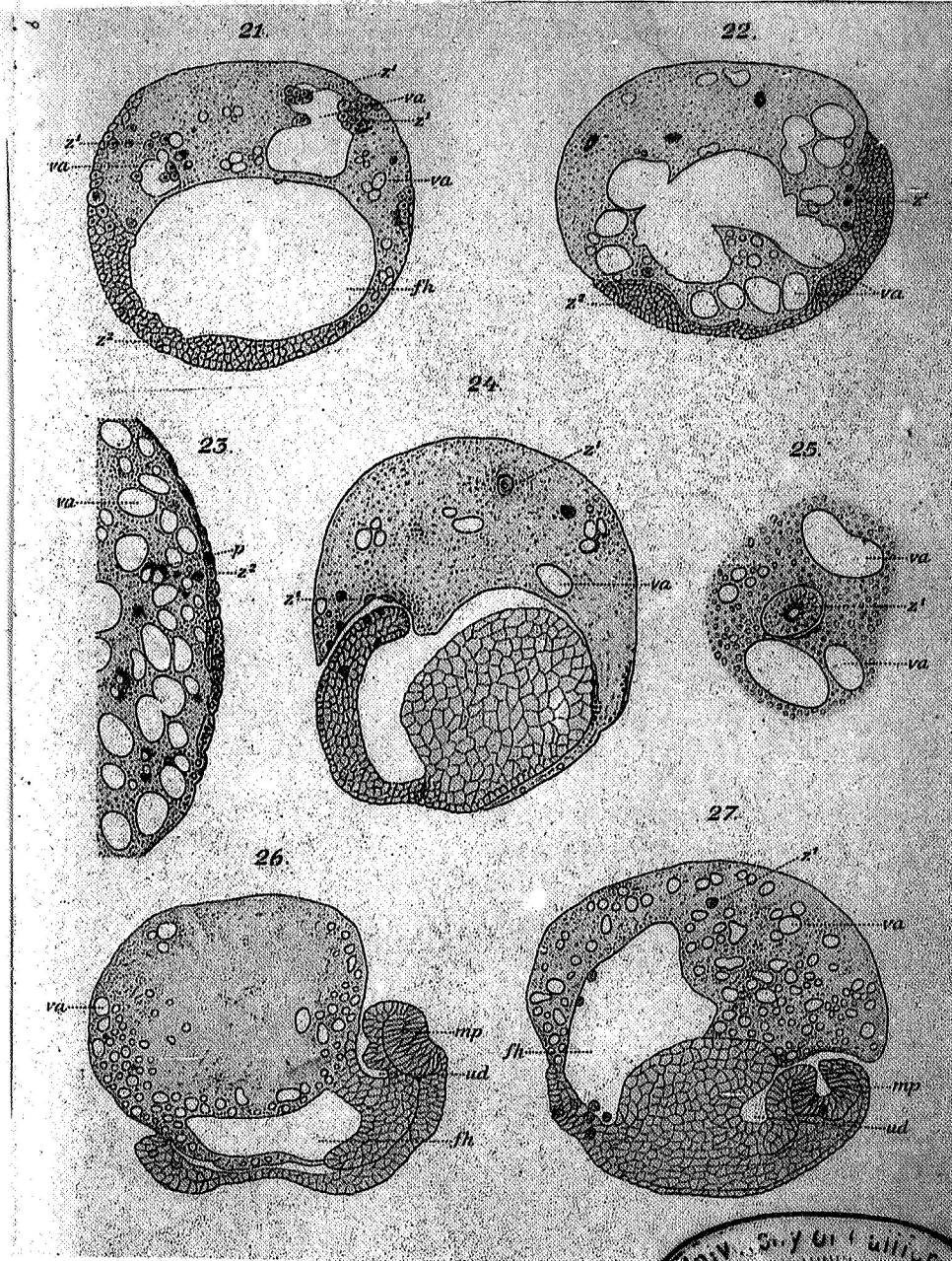
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